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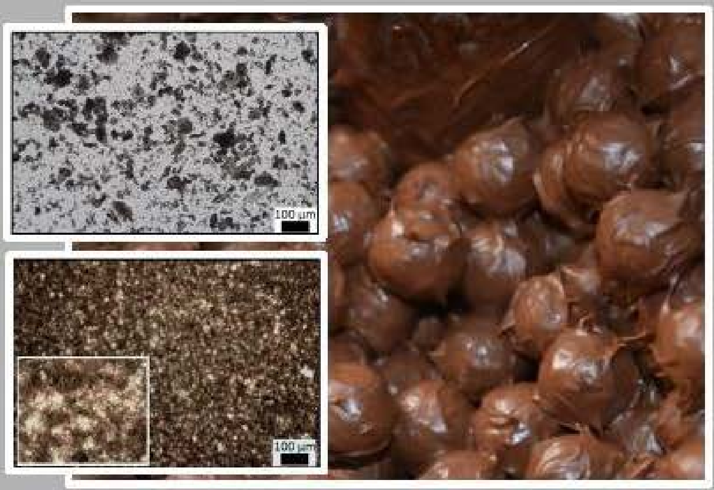

STRUCTURE-FUNCTION RELATIONS OF PALM SAP SUGAR IN DARK CHOCOLATE

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SAMENVATTING

Chocolade is een van de meest populaire zoetwarenproducten ter wereld dankzij zijn unieke smaaksensatie die we ervaren tijdens de consumptie ervan. Chocolade wordt overwegend in ontwikkelde landen verbruikt, alhoewel cacao als grondstof van chocolade wordt geproduceerd in ontwikkelingslanden zoals de Ivoorkust, Ghana en Indonesië. In de cacao-producerende landen is het chocoladeverbruik echter relatief laag. Het feit dat ongeveer 90-95% van de cacao ter wereld wordt geproduceerd door kleine boeren beperkt de mogelijkheid dat cacaoboeren hun eigen chocolade zouden produceren, wegens de onbetaalbare prijs voor de grote chocoladeverwerkingsinstallaties. Daarnaast kan deze vaststelling ook worden geassocieerd met het gebrek aan kennis.

In donkere chocolade kan het suikergehalte tot 50% bedragen. Gezien deze hoge hoeveelheid in de chocoladematrix, bepaalt suiker niet alleen de zoetheid, maar ook de reologische en texturele eigenschappen. Tegenwoordig worden inspanningen om sucrose, als de meest voorkomende chocolade zoetstof, geheel of gedeeltelijk te vervangen steeds populairder. Het hoofddoel ervan is om chocolade met een lagere caloriewaarde en glycemische index te creëren. Sucrose vervangen is echter een grote uitdaging. Het gebruik van alternatieve zoetstoffen resulteert immers vaak in verschillende chocoladekenmerken. Een soort zoetstof die potentieel gebruikt kan worden in chocolade is palmsapsuiker. Deze suiker wordt veel gebruikt als een traditioneel en alternatief zoetmiddel voor dranken en voedingsmiddelen in de Zuidoost-Aziatische en Zuid-Aziatische regio's. Het bepaalt niet alleen de zoetheid van de producten, maar ontwikkelt ook hun kleur, aroma en smaak. Vandaar dat met het gebruik van palmsapsuiker als zoetstof, chocolade met unieke eigenschappen kan worden gecreëerd.

Om chocolade van hoge kwaliteit te kunnen creëren die is geformuleerd met palmsapsuiker, werd een uitgebreid onderzoek uitgevoerd naar de fysicochemische eigenschappen van palmsapsuiker en diens rol bij het bepalen van de kwaliteitskenmerken van chocolade. Daarnaast werd ook een studie omtrent kleinschalige chocoladeverwerkingsmethodes uitgevoerd, gezien het feit dat een kleinschalig chocoladeverwerkingssysteem meer haalbaar is in ontwikkelingslanden. Algemeen gesproken waren de objectieven van deze studie om de

functionaliteit van palmsapsuiker in donkere chocolade te onderzoeken, en om de kennis van kleinschalige chocoladeproductiesystemen te verbeteren.

Het eerste deel (**hoofdstuk 1**) van het proefschrift geeft een overzicht van de vervaardiging van chocolade. De nadruk wordt gelegd op de ingrediënten van donkere chocolade (suiker, cacaoboter, cacaomassa / cacaopoeder, dispergeermiddel) en hun functionaliteit. Daarnaast worden de cacao- en chocoladeverwerkingssystemen (van cacaobonen tot tussenproducten, en van tussenproducten tot chocolade) beknopt beschreven. Kwaliteitsparameters van chocolade, zoals fijnheid, reologisch gedrag, uitzicht, thermisch gedrag, aroma profiel en hun beïnvloedende factoren worden ook overzichtelijk en kritisch beschreven.

In **hoofdstuk 2** worden de eigenschappen van sucrose, als de meest voorkomende chocolade zoetstof, en zijn functionaliteit in donkere chocolade grondig besproken. Daarnaast wordt een overzicht gegeven van de alternatieve zoetstoffen die in chocolade worden gebruikt, zoals laag-verteerbare koolhydraten, bulkzoetstoffen en zoetstoffen met een hoog-intensieve zoetstoffen. Hun effect op het reologisch gedrag, de texturele eigenschappen, het uitzicht en de smelteigenschappen van chocolade worden ook besproken. Vervolgens beschrijft dit hoofdstuk het profiel van palmsapsuiker, inclusief de soorten (siroop, gegoten en grove suiker), de verwerking en de samenstelling ervan, alsmede de factoren die de kwaliteit van palmsapsuiker beïnvloeden.

Om de eigenschappen van palmsapsuiker beter te begrijpen, met name die welke invloed hebben op de kwaliteitsattributen van chocolade, werden meerdere fysicochemische karakterisaties uitgevoerd. De resultaten worden voorgesteld en besproken in **hoofdstuk 3**. Het blijkt dat palmsapsuikers (kokos- en palmsuiker) relatief hoge gehaltes aan vocht (1.0 - 2.4%), eiwitten (0.5% - 1.6%), fructose (1.3% - 3.3%), glucose (0.7% - 2.0%) en as (0.3% - 2.1%) bevatten. Door de aanwezigheid van deze onzuiverheden waren de smelt- en glastransitietemperatuur van palmsapsuikers lager dan die van zuivere sucrose. Daarnaast was de partikeldensiteit van palmsapsuikers ook lager dan die van sucrose (1.52-1.56 g/cm^3). De grootte van de partikels van palmsapsuikers waren was ook minder uniform dan die van sucrose. De variaties in de fysicochemische eigenschappen van palmsapsuikers kunnen worden toegeschreven aan de variatie van het sap of de nectar dat gebruikt wordt als grondstof, en aan de verwerkingsmethoden die door de suikerproducenten worden gebruikt.

In **hoofdstuk 4** wordt een onderzoek voor een alternatieve verwerkingsmethode van chocolade, die economisch toegepast kan worden door kleine chocoladeproducenten in cacao-producerende landen, besproken. Een combinatie van een kogelmolen met Stephan menger, ook als vochtverwijderings- en vloeibaarmakingsapparaat, werd gebruikt om donkere chocolade te produceren. Sucrose en twee verschillende cacaobronnen, namelijk cacaomassa en cacaopoeder, werden gebruikt als de ingrediënten om de mate van effectiviteit van dit verwerkingssysteem te begrijpen bij het creëren van hoogwaardige chocolade in termen van reologisch gedrag. De belangrijkste experimentele variabelen in dit onderzoek waren de lecithineconcentratie, kogelgrootte en de tijd van het vermalen. Al deze variabelen en hun interacties beïnvloedden de reologische eigenschappen van de chocolade sterk. Daarna werd de kennis over de performantie van deze alternatieve verwerkingsmethode toegepast om chocolade gezoet met palmsapsuiker te produceren (**hoofdstuk 5**). Als referentie werd ook chocolade gezoet met palmsapsuiker geproduceerd met de conventionele verwerkingsmethode. In dit hoofdstuk wordt de functionaliteit van palmsapsuiker in de kwaliteitskenmerken van donkere chocolade onderzocht in termen van fijnheid, reologisch gedrag en aromaprofiel. Uit deze studie bleek dat het gebruik van een alternatieve verwerkingsmethode een chocolade creëerde met een vrij hoge graad van agglomeratie en met hoge viscositeit in vergelijking met chocolade die werd geproduceerd door middel van de conventionele verwerkingsmethode. Met betrekking tot de aromaprofilering resulteerde de alternatieve verwerkingsmethode in de aanwezigheid van myrceen, ß-trans-ocimeen en isoamylacetaat, stoffen die niet in de conventioneel geproduceerde chocolade werden waargenomen.

Om het beste inzicht te krijgen in de functionaliteit van palmsapsuiker in donkere chocolade geproduceerd door middel van de alternatieve verwerking, werden 5 soorten palmsapsuikers, met verschillende fysicochemische eigenschappen en vervaardigd uit kokosnoot en palmsap, gebruikt om sucrose als chocolade zoetstof te vervangen (**hoofdstuk 6**). De kwaliteitskenmerken van de onderzochte chocolade waren kleur, hardheid, fijnheid, vloeigedrag en aromaprofiel. De aanwezigheid van een relatief hoog vochtgehalte alsook glucose en fructose als hygroscopische materialen in palmsapsuiker veroorzaakte het agglomereren van partikels, wat op zijn beurt de interacties tussen partikels beïnvloedde. Hun combinatie met de lage partikeldichtheid van palmsapsuiker resulteerde in een chocolade met een lichtere kleur, hogere hardheid en hogere viscositeit. Met betrekking tot het aroma bevatte chocolade gezoet met palmsapsuiker hoge concentraties pyrazine-gebaseerde verbindingen, evenals de aanwezigheid van 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on (DDMP) die niet in sucrose gezoete donkere chocolade werd gevonden.

Zoals in hoofdstuk 2 en 3 besproken, wordt palmsapsuiker nog steeds traditioneel geproduceerd door boeren, wat resulteert in niet-gestandaardiseerde fysicochemische eigenschappen. Om aldus beter inzicht te krijgen in de functionaliteit van de verbindingen die aanwezig zijn in palmsapsuiker, werden de reologische, texturele en microstructurele eigenschappen van donkere chocolade grondig onderzocht door gedeeltelijke vervanging van sucrose door palmsapsuiker (**hoofdstuk 7**). Vijf sucrose-palmsuiker mengsels met verschillende palmsapsuiker verhoudingen werden gebruikt als chocolade zoetstoffen. Het aroma profiel en het uiterlijk aspect van donkere chocolade werden ook op dezelfde manier onderzocht (**hoofdstuk 8**). De resultaten toonden aan dat in het algemeen de Casson vloeigrens afnam en de Casson plastische viscositeit toenam naarmate het aandeel van palmsapsuiker toenam. Terwijl de hardheid significant toenam door de toevoeging van palmsapsuiker tot 50%, ging deze uitvlakken bij hogere palmsuikerverhoudingen. Deze resultaten in het vloeigedrag en de hardheid van de chocolade werden beïnvloed door de combinatie van vocht, de aanwezigheid van reducerende suikers, de partikelgroottedistributie en de partikeldensiteit van de palmsapsuiker. Het aroma profiel van chocolade die is gezoet met palmsapsuiker wordt sterk beïnvloed door het aromaprofiel van palmsapsuiker en de amorfe suiker die aanwezig is in de chocoladematrix.

Het agglomereren van partikels in chocolade, veroorzaakt door de aanwezigheid van vocht en / of amorfe suiker, beïnvloedt niet alleen de korreligheid van chocolade maar ook zijn reologische eigenschappen. Bovendien kunnen andere kwaliteitsattributen van chocolade, in het bijzonder het uiterlijk en de hardheid, in mindere of meerdere mate ook worden gecorreleerd met het reologisch gedrag van chocolade omdat hun waarden beïnvloed worden door dezelfde factoren. Vandaar dat in dit werk de inspanningen om de kwaliteit van de chocolade gezoet met palmsapsuiker te verbeteren, zijn gebaseerd op de verbetering van het reologisch gedrag (**hoofdstuk 9**). De meest haalbare methoden die kunnen worden toegepast door kleinschalige chocoladefabrikanten om de viscositeit van chocolade te verbeteren, zijn het wijzigen van de lecithine concentratie en de duur van het vacuümproces. De resultaten toonden aan dat het gebruik van een 60 minuten vacuümproces zeer effectief was bij het verwijderen van vocht, wat resulteerde in een lagere viscositeit. Met betrekking tot de toevoeging van lecithine, toonde het resultaat aan dat men bij de beslissing om een bepaalde hoeveelheid lecithine toe te voegen, de specifieke toepassing van de chocolade in aanmerking zou moeten nemen. In dit werk werd met toenemende lecithine concentratie een hogere waarde van de Casson vloeigrens en een lagere Casson plastische viscositeit waargenomen.

In **hoofdstuk 10** worden algemene conclusies en aanbevelingen voor toekomstig onderzoek beschreven.

SUMMARY

Chocolate is one of the most popular confectionery products in the world due to its unique flavour and mouthfeel perceived during consumption. It is consumed mostly in developed countries, even though cocoa, as the raw material for chocolate manufacture, is highly produced in developing countries, such as Ivory Coast, Ghana and Indonesia. In the cocoa producing countries around the equator, chocolate consumption is relatively low. The fact that about 90–95% of cocoa in the world is produced by smallholder farmers limits the possibility of cocoa farmers to produce their own chocolate due to unaffordable price of huge chocolate processing machine installations. Apart from that, this phenomenon can also be associated with the lack of knowledge of chocolate processing.

In dark chocolate, sugar content can be up to 50%. Considering its high amount in the chocolate matrix, sugar not only determines the sweetness, but also the rheological and textural properties. Nowadays, efforts to fully or partially replace sucrose as the most common chocolate sweetener are becoming more popular. The main purpose is to create chocolate with a low caloric value and low glycemic index and a small amount of high potency sweeteners as well. However, sucrose replacement is very challenging. The use of alternative sweeteners frequently results in different chocolate characteristics. One type of sweetener that has potency as chocolate sweetener is palm sap sugar. This sugar is widely used as a traditional and an alternative sweetener for beverages and foods in the South East and South Asian regions. It does not only determines the sweetness of the products, but also develops their colour, aroma and taste. Hence, the use of palm sap sugar as chocolate sweetener is expected to create chocolate with distinct characteristics.

To be able to create high quality chocolate formulated with palm sap sugar, a comprehensive investigation on the physicochemical characteristics of palm sap sugar and their role in determining the quality attributes of chocolate was carried out. In addition, the study about small-scale chocolate processing method was also conducted considering that small-scale chocolate processing system is more feasible to be applied in developing countries. In general, the objectives of this study were to investigate the functionality of palm sap sugar in dark chocolate and to improve the knowledge on the small-scale chocolate production system.

The first part **(Chapter 1)** of the dissertation provides an overview of the chocolate manufacture. Emphasises were laid on the ingredients of dark chocolate (sugar, cocoa butter, cocoa mass / cocoa powder, dispersing agent) and their functionality. In addition, cocoa and chocolate processing systems (from bean to intermediate products and from intermediate products to chocolate) were also summarised. Apart from these, quality parameters of chocolate, such as fineness, rheological behaviour, appearance, thermal behaviour, aroma profile and their influencing factors were also critically reviewed.

In **Chapter 2**, the properties of sucrose and its functionality in dark chocolate were thoroughly reviewed. Furthermore, an overview of the common alternative sweeteners used in chocolate, such as low-digestible carbohydrates, bulk sweeteners and high-potency sweeteners (high-intensity sweeteners) as well as impact on the rheological behaviour, textural properties, appearance, melting properties of chocolate was also discussed. Afterwards, this chapter comprehensively describes the profile of palm sap sugar, including the types (syrup, moulded and coarse sugar), the processing, the composition and the factors influencing the quality of palm sap sugar.

In order to gain a better understanding about the properties of palm sap sugar, especially those that are predicted to have influence on the quality attributes of chocolate, several physicochemical characterisations were carried out. The results are presented and discussed in **Chapter 3.** It was revealed that palm sap sugars (coconut and palm sugar) contained relatively high moisture (1.0 - 2.4%), protein (0.5% - 1.6%), fructose (1.3% - 3.3%), glucose (0.7% - 2.0%) and ash (0.3% - 2.1%). Due to the presence of those impurities, the melting and glass transition temperature of palm sap sugars were lower than those of pure sucrose. Aside from these, the particle density of palm sap sugars was also lower than that of sucrose, in the range of 1.52 – 1.56 g/cm^3. Furthermore, the particle sizes of palm sap sugars were less uniform compared to those of sucrose. The variation in the physicochemical properties of palm sap sugars can be attributed to the variation of the sap/nectar as raw materials and the processing methods used by sugar producers.

In **Chapter 4**, an investigation on an alternative processing method of chocolate that can be economically applied by small-scale chocolate producers in cocoa producing countries is described and discussed. A combination of a ball mill with Stephan mixer was used to produce dark chocolate. Sucrose and two different cocoa sources, namely cocoa mass and cocoa powder were used as ingredients to understand the extent of the effectiveness of this processing system in creating high quality chocolate, in terms of rheological behaviour. The key experimental variables in this research were lecithin concentration, ball size and milling time. All these variables as well as their interactions highly influenced the rheological properties of the chocolates. Afterwards, the knowledge about the performance of this alternative processing method was applied to produce chocolate sweetened with palm sap sugar (**Chapter 5**). As reference, chocolate sweetened with palm sap sugar was also produced with conventional processing. In this chapter, the functionality of palm sap sugar in the quality attributes of dark chocolate were investigated in terms of fineness, rheological behaviour and aroma profile. This study showed that the use of alternative processing method created a chocolate with a rather high degree of agglomeration and viscosity as compared to chocolate produced by means of conventional processing. In regard of aroma profiling, the alternative processing method resulted in the presence of myrcene, ß-trans-ocimene and isoamyl acetate which were not observed in the conventionally produced chocolates.

In order to gain the best understanding about the functionality of palm sap sugar in dark chocolate produced using alternative processing, five types of palm sap sugars with different physicochemical characteristics, made from coconut and palm sap, were used to replace sucrose as chocolate sweetener (**Chapter 6**). The quality attributes of the chocolate investigated were colour, hardness, fineness, flow behaviour, and aroma profile. The presence of relatively high moisture as well as glucose and fructose as hygroscopic materials in palm sap sugar induced particle agglomeration that in turn influenced the particle-particle interactions. Their combination with low particle density of the palm sap sugar resulted in a chocolate with a lighter colour, higher hardness and higher viscosity. Regarding the aroma, palm sap sugar sweetened chocolates were marked with high concentrations of pyrazine based compounds as well as with the presence 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) which was not found in sucrose sweetened dark chocolate.

As discussed in Chapter 2 and 3, palm sap sugar is still traditionally produced by farmers, resulting in non-standardised physicochemical characteristics. Thus, to be able to get better insight about the functionality of the compounds present in palm sap sugar; the rheological, textural and microstructural properties of dark chocolate were thoroughly investigated by partial replacement of sucrose with palm sap sugar (**Chapter 7**). Five sucrose-palm sugar blends with different palm sugar proportions were used as chocolate sweeteners. The aroma profile and appearance of dark chocolate were also comprehensively studied by the same approach (**Chapter 8**). The results showed that, in general, the Casson yield value decreased and the Casson plastic viscosity increased as the proportions of the palm sap sugar increased. While the hardness was significantly increased with the addition of palm sugar up to 50%, but levelled off at a higher palm sugar proportions. These results of the flow behaviour and hardness of the chocolates were influenced by the combination of moisture, reducing sugar, particle size distribution and particle density of the palm sugar. Regarding the aroma profile, the aroma profile of palm sap sugar sweetened chocolate is highly influenced by the aroma profile of palm sap sugar and the amorphous sugar present in the chocolate matrix.

The formation of particle agglomeration in chocolate, induced by the presence of moisture and/or amorphous sugar, does not only affect the grittiness of chocolate but also its rheological properties. Furthermore, other quality attributes of chocolate, particularly hardness and appearance, can to some extent also be correlated with the rheological behaviour of chocolate because their values are influenced by the same factors. Hence, in this work, the efforts to improve the quality of palm sap sugar sweetened chocolate were based on the improvement of rheological behaviour (**Chapter 9**). The most feasible methods that can be applied by small-scale chocolate makers to improve the viscosity of palm sap sugar sweetened chocolate are by modifying the lecithin concentration and the duration of the vacuum process. The results showed that the use of 60 min vacuum process was very

effective in evaporating moisture, resulting in a lower viscosity. Regarding the lecithin addition, the result showed that the decision to add certain amount of lecithin should consider the chocolate application. In this work, the higher the lecithin concentration, the higher the Casson yield value and the lower the Casson viscosity were observed.

In **Chapter 10,** general conclusions and recommendations for future studies are described.

OUTLINE OF THE RESEARCH

The functionality of chemical composition and physical properties of palm sap sugar in dark chocolate produced using a small-scale chocolate production system is described in this dissertation, entitled “Structure-function relations of palm sap sugar in dark chocolate”. This dissertation consists of five parts, namely Part 1 (Literature review), Part 2 (Chemical composition and physical properties of palm sap sugars), Part 3 (Alternative processing of chocolate), Part 4 (Functionality of palm sap sugar in dark chocolate) and Part 5 (Improving the quality of palm sap sugar-sweetened chocolate).

PART 1 - Chapter 1 presents an overview of the world cocoa production and chocolate consumption, chocolate ingredients, chocolate manufacturing process and dark chocolate quality parameters. **Chapter 2** reviews the usage of sucrose and alternative sweeteners in chocolate. Afterwards, palm sap sugar profile with its potency as chocolate sweetener is discussed. These chapters provide scientific knowledge about sugar and sugar replacement as well as chocolate processing methods.

PART 2 - Chapter 3 studies the physicochemical properties of coarse palm sap sugars that are used as chocolate sweeteners in the research. Chemical composition and physical properties of palm sap sugars from Indonesian origin expected to have influence on the quality attributes of chocolate were investigated. The findings of this work were used to discuss the results obtained in the following chapters.

PART 3 - Chapter 4 studies the impact of processing variables, namely ball size and milling time as well as lecithin concentration on the rheological and microstructural properties of sucrose- sweetened chocolate. This work was conducted to obtain insights about alternative processing method before using it to produce chocolate formulated with palm sap sugar (**Chapter 5**). In these studies, the performance of alternative processing method was compared to the performance of the conventional one.

PART 4 - Chapter 6 studies the functionality of several types of palm sap sugars in the quality attributes of dark chocolate, followed by a thorough investigation on the rheological, textural and microstructural properties of dark chocolate by partially replacing sucrose with palm sap sugar (**Chapter 7**). A more detailed research about the aroma profile and appearance of dark chocolate formulated with palm sap sugar - sucrose blends is reported in **Chapter 8**. In these work packages, the functionality of palm sap sugar in dark chocolate were comprehensively studied. Nonetheless, a study on the improvement of the quality attributes of chocolate, more particularly on the fineness and rheological behaviour, was not conducted.

PART 5 - Chapter 9 deals with the efforts to improve the quality of palm sap sugar-sweetened chocolate, in terms of fineness and rheological behaviour. This part focuses on the process adaptation of the studied small-scale production system and fine-tuning of lecithin concentration. At the end**,** general conclusions are drawn and recommendations for future studies are proposed in **Chapter 10.**

The layout of this PhD research is summarised in Figure I.

STRUCTURE-FUNCTION RELATIONS OF PALM SAP SUGAR IN DARK CHOCOLATE

PART 1 **Literature review**	• **Chapter 1** : Chocolate manufacture • **Chapter 2** : Sucrose replacement : an introduction to palm sap sugar
PART 2 **Chemical composition and physical properties of palm sap sugars**	• **Chapter 3** : Physicochemical properties of coarse palm sap sugars from Indonesian origin
PART 3 **Alternative processing of chocolate**	• **Chapter 4** : Impact of processing variables on the rheological behaviour and microstructural properties of dark chocolate produced by the combination of a ball mill and a liquefier device • **Chapter 5** : Feasibility of a small-scale production system approach for palm sugar sweetened dark chocolate
PART 4 **Functionality of palm sap sugar in dark chocolate**	• **Chapter 6 :** Quality attributes of dark chocolates formulated with palm sap sugar as natural alternative sweetener • **Chapter 7 :** Investigating the rheological, microstructural and textural properties of chocolates sweetened with palm sap-based sugar as a partial replacement of sucrose • **Chapter 8 :** Aroma profile and appearance of dark chocolate formulated with palm sugar - sucrose blends
PART 5 **Improving the quality of palm sap sugar-sweetened chocolate**	• **Chapter 9** : Feasible approaches for small-scale chocolate producer to improve the flow behaviour of chocolate formulated with palm sap sugar

Chapter 10 : General Conclusions and Future Perspectives

Figure I. Scheme of the PhD research

LIST OF ABBREVIATIONS

η_{CA}	: Casson plastic viscosity
τc_{A}	: Casson yield value
$\dot{Y}$	: Shear rate
τ	: Shear stress
ANOVA	: Analysis of variance
AP	: Alternative processing
CP	: Conventional processing
CCS1	: Coarse coconut sugar 1
CCS2	: Coarse coconut sugar 2
CPS1	: Coarse palm sugar 1
CPS2	: Coarse palm sugar 2
CPS3	: Coarse palm sugar 3
CS	: Coarse sucrose
CTU	: Chocolate temper unit
DDMP	: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
DSC	: Differential scanning calorimeter
FS	: Fine sucrose
GI	: Glycemic index
HS–SPME–GC–MS	: Headspace - solid phase micro extraction - gas chromatography - mass spectrometry
ICCO	: International Cocoa Organisation
LVR	: Linear viscoelastic region
OAV	: Odour activity value
OTV	: Odour threshold value
PCA	: Principal component analysis
PGPR	: Polyglycerol polyricinoleate
POP	: 1,3-dipalmitoyl-2-oleoyl-glycerol
POS	: rac-palmitoylstearoyl-2-oleoyl-glycerol
PSD	: Particle size distribution
SEM	: Scanning electron microscope

SOS : 1,3-stearoyl-2-oleoyl-glycerol

TAG : Triacylglycerol

CHAPTER 1

CHOCOLATE MANUFACTURE

CHAPTER 1. CHOCOLATE MANUFACTURE

1.1 INTRODUCTION

Chocolate is one of the most popular confectionery products in the world due to the pleasure derived upon its consumption which is associated with its distinguishable flavour and mouthfeel (Afoakwa, 2010; Aidoo et al., 2014; Beckett, 2009; Lipp and Anklam, 1998). Growing consumer demand for chocolate and its derivative products is attributed to those characteristics (Afoakwa, 2010; Beckett, 2009). Recently, the demand for various chocolate-coated products, such as crackers and ice creams as well as other products containing cocoa powder, such as beverages and cakes is increasing (Afoakwa, 2016; Aidoo, 2015).

Chocolate can be classified into 3 different types, namely dark chocolate, white chocolate, and milk chocolate (Afoakwa, 2010; Beckett, 2009; Fernandes et al., 2013; Glicerina et al., 2013). Dark chocolate is made from sugar and cocoa solids dispersed in cocoa butter with the addition of a minute amount of lecithin. This type of chocolate has a very dark colour and a bitter taste. White chocolate is produced without adding cocoa solids, creating chocolate with white colour and a pronounced sweet and milky taste. The absence of cocoa solids reduces its shelf-life to some extent (Beckett, 2009). Milk chocolate, on the other hand, has the most complete ingredients, namely cocoa solids, milk powder, sugar, cocoa butter and lecithin.

Cocoa as the main ingredient for chocolate production is derived from the beans of cacao tree (*Theobroma cacao L.*) which can only grow at ± 20° north and south of the equatorial line. In this region, temperature, soil condition, humidity and level of rainfall are appropriate for plant growing (Adewole et al., 2011; Fowler, 2009; Kongor et al., 2016; Ololade et al., 2010). In general, there are three varieties of cacao which are commonly grown, namely *Forastero*, *Criollo*, and *Trinitario*. *Forastero* is the main variety planted in the world (more than 95%). It is highly cultivated in West Africa and South East Asia and has a strong cocoa flavour (Afoakwa, 2016). *Criollo*, commonly grown in Central America, is well known to have the most aromatic flavour quality (Afoakwa, 2016). Meanwhile, *Trinitario* is a hybrid between the *Criollo* and *Forastero* variety which has strong basic chocolate characters and some typical winery aroma (Afoakwa et al., 2008c; Fowler, 2009).

1.2 WORLD COCOA PRODUCTION AND CHOCOLATE CONSUMPTION

In the world, irrespective of the cacao variety, cacao is abundantly planted in three regions, namely West Africa, Central and South America as well as South East Asia (Afoakwa, 2016; Fowler, 2009). As cited by Afoakwa (2016), ICCO (2015) reported that Africa, as the largest cocoa supplier in the world, supplies about 72.3% of world cocoa exports, America (16.7%) and Asia-Oceania (11.0%). At a country level, Ivory Coast is the largest cocoa exporter in the world, contributing up to 40.6% of global exports, followed by Ghana (19.2%) and Indonesia (8.9%). In 2016, about 95% of world cocoa production (about 4.23 million) was supplied by the eight largest cocoa-producing countries, namely Ivory Coast, Ghana, Indonesia, Nigeria, Ecuador, Cameroon, Brazil and Malaysia (in descending order).

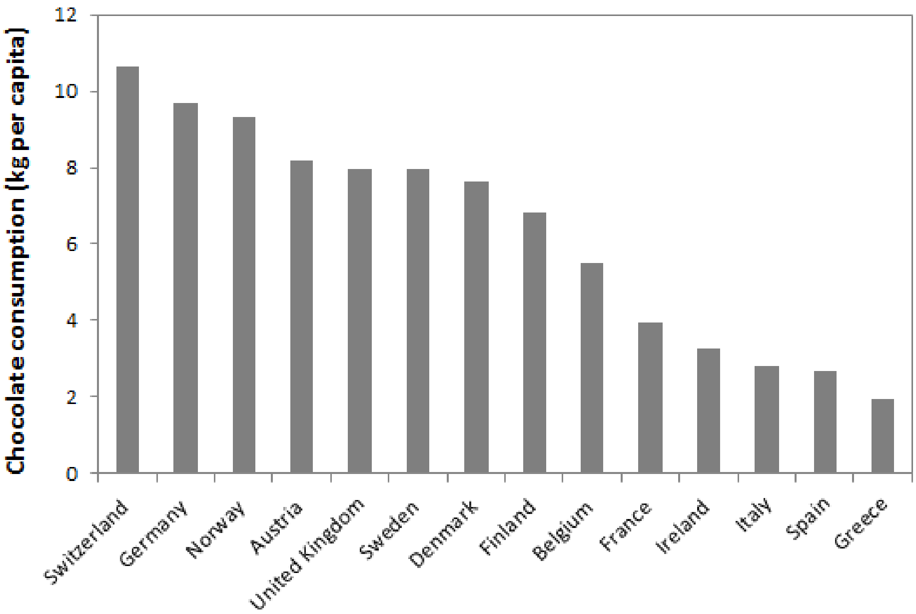


Figure 1.1. Consumption of chocolate products (kg per capita) in 2012 (Afoakwa, 2016)

Chocolate is consumed mostly in the developed countries, such us in European countries mainly due to differences in typical diet and traditional habits. As reported by Afoakwa (2016), Switzerland is a country with the highest chocolate consumption per capita in 2012, followed by Germany, Norway, Austria, and United Kingdom. From this report, it can be remarked that chocolate consumption (kg per capita) in the northern European countries, especially those with a strong chocolate tradition, such as Switzerland, United Kingdom, Belgium, Germany and Ireland tends to be higher than the other countries (Figure 1.1). However, it can be noticed that there are no cocoa producing countries in the list of countries with high chocolate consumption showing that either the temperature is too warm, hence not suitable for the chocolate market or the inability of cocoa farmers to produce their own chocolate due to unaffordable price of chocolate processing machinery. The latter factor is caused by the fact that about 90–95% of cocoa world production is produced by smallholder farmers with relatively low income (Afoakwa, 2016).

1.3 DARK CHOCOLATE INGREDIENTS

Dark chocolate is a dense suspension of solid particles containing about 65-75% sugar and cocoa solids dispersed in cocoa butter which is solid at ambient temperature (around 25 ^{o}C) and melts close to body temperature (37^{o}C) (Afoakwa et al., 2008a; Afoakwa et al., 2008c; Afoakwa et al., 2008d; De Graef et al., 2011; Fernandes et al., 2013; Glicerina et al., 2013).

1.3.1 Sugar

The most common sugar used in chocolate manufacturing is sucrose (Afoakwa, 2016; Beckett, 2009), which can be utilised up to 50%, depending on the chocolate formulation (Aidoo et al., 2013; Belscak-Cvitanovic et al., 2015; Mathlouthi and Reiser, 1995). Considering its high amount in the chocolate matrix, sucrose highly determines the chocolate characteristics, mainly the rheological and textural properties. Studies have shown that sucrose is uniquely suited to be used in chocolate manufacturing which requires a dry sweetener with low hygroscopicity (Mathlouthi and Reiser, 1995). Dry sugar is preferable because the presence of moisture causes the sugar particles to stick together, which further increases the chocolate viscosity. The detailed information about sucrose properties and its functionality as well as its alternative as chocolate sweetener is described in Chapter 2.

1.3.2 Cocoa butter

Cocoa butter, commonly present in the amount of 25-35%, is a dark chocolate ingredient responsible for dispersing the other ingredients (Afoakwa et al., 2007; Lipp and Anklam, 1998). It mainly determines the textural and rheological properties, glossiness, snap as well as melting behaviour of chocolate (De Clercq et al., 2016). Compared to other fats, cocoa butter has a relatively simple triacylglycerol (TAG) composition with the main TAG being 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), rac-palmitoylstearoyl-2-oleoyl-glycerol (POS) and 1,3-stearoyl-2-oleoyl-glycerol (SOS) (Figure 1.2) (Afoakwa et al., 2007; Campos et al., 2009; De

Clercq et al., 2016). This composition highly relies on the cocoa bean origin and growing conditions, but in average, POS, SOS and POP are present in the amount of around 35%, 23% and 15 %, respectively (De Clercq et al., 2016). This rather unique TAG composition ensures chocolates with a melting temperature in the range of 23-37^{o}C (Afoakwa et al., 2007). This specific melting behaviour creates a distinctive 'mouthfeel' when a high-quality chocolate is consumed (Lipp and Anklam, 1998). Changes in the TAG composition affect its crystallisation behaviour which further influences its functionality (Campos et al., 2009). With regard to the crystallisation behaviour, cocoa butter exhibits a relatively complex polymorphism (Delbaere et al., 2016) which is defined as the capability of a molecule to crystallise in different crystal packing configurations (Talbot, 1994). The detailed information about polymorphism is described in Section 1.4.3.1**.**

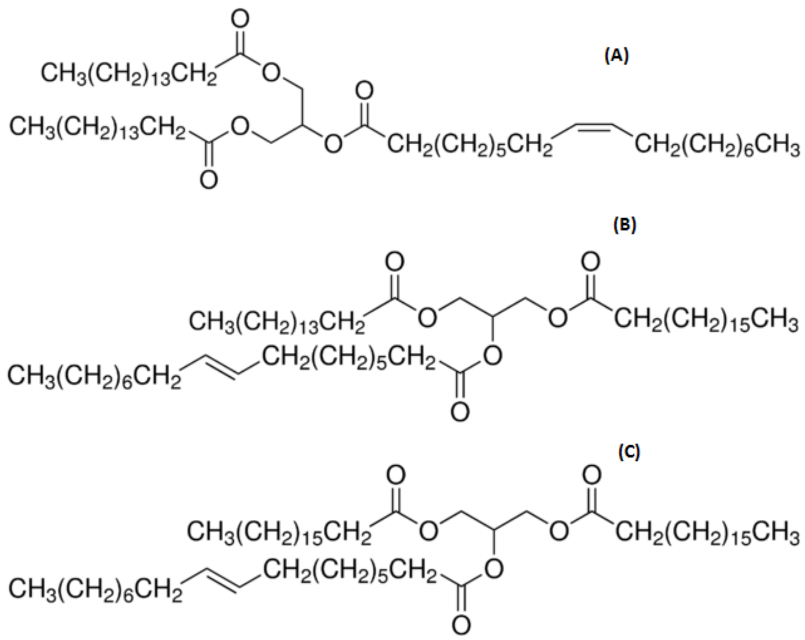


Figure 1.2. Chemical structures of (A) 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), (B) rac-palmitoylstearoyl-2-oleoyl-glycerol (POS) and (C) 1,3-stearoyl-2-oleoyl-glycerol (SOS)

1.3.3 Cocoa mass and cocoa powder

Cocoa mass is a main chocolate ingredient produced by grinding roasted cocoa nibs to a liquid-like pasty mass. To produce cocoa powder, cocoa mass is pressed to squeeze out a large part of the cocoa butter which is then formed into a solid material known as cocoa butter cake. Milling is then needed to break down the cake into powdered material (Afoakwa, 2010; Kamphuis, 2009; Meursing, 1994). For the cocoa powder production, the cocoa nibs can undergo an alkalisation process in order to create a darker product

(Kamphuis, 2009; Rodríguez et al., 2009) and to improve the dispersibility of the cocoa solids in water (Afoakwa, 2016). Cocoa butter is present in the range of 50-58% in cocoa mass, depending on the variety and growing conditions of the cacao tree, while in cocoa powder, it is present in the range of 10-24% (Kamphuis, 2009; Meursing, 1994).

In the chocolate matrix, cocoa mass acts as a bulking and texturing agent as well as flavour developer. However, in certain cases, its role can be replaced by cocoa powder, for instance, in compound chocolates (Meursing, 1994). In the market, cocoa mass from different regions, especially from West Africa are commonly blended (Kamphuis, 2009), thus rather uniform physicochemical characteristics of the cocoa mass are obtained. In contrast, single-origin chocolates, produced from cocoa beans from one location or one country of origin, is gaining popularity (Kamphuis, 2009). In single-origin chocolate production, the chocolates are specially made from fine cocoa varieties, which have specific flavours. Due to a high degree of fat saturation (Afoakwa et al., 2007; De Clercq et al., 2016) and the presence of natural antioxidants (Muhammad et al., 2017; Othman et al., 2007), cocoa mass has a long shelf life and can be stored for several weeks in a molten form/state or for more than a year in a solid form (Kamphuis, 2009). Cocoa powder can also be stored for a long time, if it is well packaged (Meursing, 1994).

1.3.4 Dispersing agent

Surface properties of solid particles in chocolate determine the way the particles interact with each other and with the cocoa butter phase (De Graef et al., 2011; Rousset et al., 2002). In the chocolate matrix, sugar is widely known as a hydrophilic or a lipophobic ingredient. Thus, a dispersing agent is required to lower the interfacial tension between sugar particles and cocoa butter phase (Schantz and Rohm, 2005b). The main purpose of using dispersing agents in chocolate is to maintain the flow behaviour. Some potential dispersing agents which can be used are lecithin, polyglycerol polyricinoleate (PGPR) and YN (synthetic lecithin) (Afoakwa et al., 2007; Chevalley, 1994; Schantz and Rohm, 2005b).

The most common dispersing agent applied in chocolate is lecithin, which has been used since 1930. It is usually extracted from the by-product of soybean oil production (Afoakwa, 2010; Beckett, 2008; Rousset et al., 2002). Lecithin consists of a polar head group which is able to attach to the sugar and lipid nonpolar tails which extend in the fat system to facilitate the flow, to some extent (Beckett, 2008, 2009). It was reported by Beckett (2008) and Chevalley (1994) that an addition of 0.3% lecithin into chocolate equals to the use of ten times this weight of cocoa butter. However, at a higher lecithin concentration, *e.g.* above 0.5%, the yield value of chocolate with a fat content of 33.5% increases as the lecithin was increased, while the plastic viscosity decreases. A similar observation was reported by De Graef et al. (2011), in which the Casson yield value of chocolate with a fat content of 36.7% was reported to increase at lecithin concentrations higher than 0.4%. This phenomenon is due to the formation of reverse micelles in cocoa butter and/or the formation of self-association of lecithin possibly as multilayers around sugar (Afoakwa et al., 2007; Beckett, 2009). Nevertheless, in the case of relatively low fat content (below 30%), the use of a higher concentration of lecithin can be considered. In some cases, in order to have better efficiency, different types of dispersing agents are mixed at certain ratio (Schantz and Rohm, 2005b).

During manufacturing, lecithin should be added at the correct moment. The addition of lecithin at the beginning of the conching process reduces its effectiveness in decreasing the viscosity of chocolate. This phenomenon occurs due to the fact that if lecithin is added too early at the beginning of conching, some of the lecithin is absorbed by the solid particles, thus reducing its efficiency. Apart from this, exposure to relatively high temperatures for a long time also reduces lecithin efficacy (Chevalley, 1994)

1.4 COCOA AND CHOCOLATE PROCESSING

In order to produce high quality chocolate bars, cocoa beans as the main raw material for chocolate production undergo a sequence of processes, including beans to cocoa mass processing (fermentation, drying, roasting, de-shelling, and grinding), cocoa mass to molten chocolate processing (either by conventional or alternative processing), and molten chocolate to chocolate bar shaping (tempering, moulding and crystal maturation). The first sequence is very crucial in generating high quality cocoa ingredients, namely cocoa mass, cocoa powder and cocoa butter. The second phase is responsible for turning high quality cocoa into high quality molten chocolate with good flavour and desired rheological characteristics. The third stage has a role in defining the textural characteristics, melting behaviour of chocolate upon consumption and heat stability during storage.

1.4.1 From cocoa beans to cocoa mass, cocoa powder and cocoa butter

After the cacao pods are harvested from the trees, they are directly opened to collect the beans or undergo pod storage for few days. The latter option has been reported to have an important role in the flavour development of the beans during subsequent fermentation and processing (Afoakwa, 2010; Afoakwa et al., 2013; Baker et al., 1994; Meyer et al., 1989; Nazaruddin et al., 2006).

Basically, the sequence of cocoa beans processing consists of fermentation, drying, roasting, de-shelling/breaking and grinding, consecutively. However, an optional process, called alkalisation is also commonly done on cocoa nibs which is predominantly used for the production of cocoa powder. This process mainly aim to modify the colour and flavour as well as to improve dispersibility of the cocoa solids in water (Afoakwa, 2010; Kamphuis, 2009).

1.4.1.1 Fermentation

Fermentation is the first post-harvest handling performed to the beans. This process is very essential for the development of appropriate flavours precursors, which takes about 5 to 6 days for the *Forastero* variety and 1–3 days for the *Criollo* variety. At the farmer level, fermentation of cocoa beans is commonly done by using heaps and boxes (Afoakwa, 2010; Fowler, 2009). Variation in the fermentation duration leads to different chemical characteristics of final product, such as acidity (Bonvehi, 2005; Frauendorfer and Schieberle, 2008; Kongor et al., 2016).

Due to its sugar content (10–15%), the pulp is an ideal substrate for the growth of micro-organisms that naturally come from the environment once the cocoa beans have been opened. There are 3 stages of the fermentation process, although, in practice overlapping reactions can occur due to variety of the micro-organisms among different regions (Fowler, 2009). In the first stage (24-26 hours), sugar is converted into alcohol by yeasts under low oxygen and low pH (less than 4). This stage will inhibit germination and contribute to the structural changes of cocoa. The beans become inactive on the second day of fermentation, due to the presence of acetic acid and alcohol. In the second stage (48-96 hours), the conversion of sugar and some organic acids into lactic acid by lactic acid bacteria occurs. In the last stage, the degree of aeration increases and acetic acid bacteria become more dominant. They convert alcohol into acetic acid in a strong exothermic reaction, thus increasing the temperature up to 45-50°C.

When the beans "die" during fermentation, the cell walls and membranes will break down. These lead to the reactions of various compounds and enzymes (Fowler, 2009), such as colour changes due to hydrolysis of phenolic components by glycosidases (Afoakwa et al., 2008c), an increase in volatile compounds, such as alcohols, organic acids, esters and aldehydes (Frauendorfer and Schieberle, 2008), and a decrease in the bitterness and astringency due to oxidation and polymerization of phenolic compounds (Kongor et al., 2016; Misnawi et al., 2004). Apart from these, free amino acids and peptides, which are flavour precursors, are generated from enzymatic degradation of cocoa proteins and sucrose, respectively (Frauendorfer and Schieberle, 2008; Kongor et al., 2016; Krähmer et al., 2015).

1.4.1.2 Drying

Drying of cocoa beans is required once the fermentation has finished to prevent mould growth during storage. This process results in cocoa beans with moisture contents of approximately 6-8% (Afoakwa, 2010; Fowler, 2009; Hancock and Fowler, 1994). Cocoa bean drying can be done by sun drying or artificial drying techniques. In the first method, cocoa beans are spread out on mats, trays or terraces on the ground. This method is cost-effective and can produce high quality beans. In the latter techniques, infrared light, rotary hot air dryer or static air-ventilated oven can be used (Fowler, 2009; Guehi et al., 2010). The simplest drying method that can be easily applied by farmers uses hot air produced from wood fire. The drawback of this method is that shell hardening may occur due to fast drying. Thus, volatiles acids are trapped inside the beans, resulting in more acidic beans. In addition, smoky bean may be also formed (Fowler, 2009; Hancock and Fowler, 1994).

During drying of fermented cocoa beans, major polyphenol oxidizing reactions are catalysed by polyphenol oxidases. This process does not only reduce the bitterness and astringency of cocoa, but also develop the brown-coloured beans (Afoakwa et al., 2008c). In addition, reducing sugars also participate in the non-enzymatic browning reactions, Maillard reaction, which form volatile fractions of pyrazines (Kongor et al., 2016). Well-dried beans exhibit the desired brown colour as well as low astringency and bitterness without the presence of off-flavours (Afoakwa, 2010)

1.4.1.3 Debacterisation

Cocoa bean is a potential source of pathogens, such as Salmonella and Coliforms. They can be present in cocoa during fermentation and drying process due to lack of environmental control (Beckett, 2009; Izurieta and Komitopoulou, 2012). Hence, after drying, debacterisation step is highly needed. The debacterisation step can be carried out in a batch or continous system by combining heat and water to create steam which ensures that all the pathogenic bacteria are destroyed (Afoakwa, 2016). In industrial level, this step can be done either before or after roasting process. When the debacterisation is conducted after the roasting process, debacterisation can be done by injecting a fine water spray of steam into the roasting chamber for about 20 seconds (Awua, 2002).

1.4.1.4 Roasting

Roasting of fermented cocoa beans aims to develop the cocoa flavour, reduce moisture and acidity as well as to release the beans from the shell (Afoakwa, 2010; Ziegleder, 2009). Aside from this, reduction in the number of micro-organisms occurs, thus food-grade products, such as cocoa butter, cocoa powder and cocoa mass can be obtained (Afoakwa, 2010). Cocoa beans are usually roasted at temperatures between 130^{o}C and 150^{o}C for 15-45 min (Krysiak, 2006). Batch drum roaster is frequently used for cocoa bean roasting, applying the heat obtained from the drum wall. The drum tumbles the beans so that localised overheating is avoided (Kamphuis, 2009). Before roasting, pre-drying is commonly applied to decrease the water content to less than 4%. Without pre-drying, cocoa would generate a more cooked aroma. During roasting, flavour precursors namely free amino acids and reducing sugars produced during fermentation and drying process undergo the Maillard reaction. This reaction generates desirable flavour compounds such as pyrazines, alcohols, esters, aldehydes, ketones, furans, thiazoles, pyrones, acids, imines, amines, oxazoles, pyrroles and ethers. After roasting, cocoa will possess the typical cocoa aroma with low acidity (Counet et al., 2002b; Frauendorfer and Schieberle, 2008; Jinap et al., 1998; Kongor et al., 2016; Krysiak, 2006; Serra Bonvehí and Ventura Coll, 2002; Ziegleder, 2009).

1.4.1.5 Grinding and pressing: cocoa mass, cocoa powder and cocoa butter production

Prior to the grinding process, breaking of the beans and winnowing are imperative since undesirable contaminants which can create off-flavour may be found in the shell. These processes ensure that the cocoa nibs are well broken and properly deshelled and separated. Cleaned cocoa nibs are collected to undergo further cocoa processing, while the shells are then discarded for animal feed / fertiliser or bio-energy purposes (Afoakwa, 2010; Kamphuis, 2009).

Cocoa mass is manufactured by grinding the cocoa nibs, while cocoa powder and cocoa butter are produced by separating the liquid (fat) and solid part of the cocoa mass. The fineness to which the mass has to be ground depends on its final use. For cocoa powder production, fine grinding is critical. Several machines which can be used to produce cocoa mass are stone mills, disc mills, hammer mills and ball mills (Afoakwa, 2016). As for microbial destruction, it can be done by heating the cocoa mass at a temperature around 90–100◦C (Awua, 2002). Cocoa butter can be obtained by pressing the cocoa mass using hydraulic press machine generating cocoa cake as by-product. The cocoa cake, depending on its residual fat content, can be classified into high fat (22-24%) and low-fat cocoa cake (10-12%). It is transformed into cocoa powder by grinding the cocoa cake using hammer-or-disc mills (Afoakwa, 2010; Kamphuis, 2009; Meursing, 1994).

1.4.2 From cocoa mass to molten chocolate

Chocolate is one of the most popular confectionery products in the world commonly produced using middle-big scale machines which require high capital investment (Afoakwa, 2010; Beckett, 2009). This is a major limitation for developing countries such as Ghana, Indonesia and Ivory Coast *etc.* where cocoa as the main raw material for chocolate production is highly produced (Afoakwa, 2016; International Cocoa Organisation (ICCO), 2014). Therefore, an alternative small-scale chocolate production system which can be economically applied by farmers and small-scale chocolate industries in cocoa producing countries is highly needed.

1.4.2.1 Conventional processing : mixing, roll-refining and conching

Conventionally, molten chocolate is produced using a sequence of mixing, roll-refining and conching (Figure 1.3) (Afoakwa, 2010; Beckett, 2009). Mixing is the first and basic operation employed using a combination of time and temperature in either a batch or continuous system. A batch mixer could be used in the small-scale chocolate production while the continuous mixer, which uses automated kneaders, is used mainly in large-scale chocolate production. The aim of mixing is to produce an intermediate product with a somewhat tough texture and plastic consistency (Afoakwa, 2010). Otherwise, the mixture will not be able to attach on the rolls perfectly during roll refining (Minifie, 1989).

Refining of the chocolate mixture is mainly aimed to reduce the particle size of the solids which can be achieved using a combination of two- and five-roll refiners (Afoakwa, 2010; Lucisano et al., 2006). Aside from this, refining also acts as a means to disperse the particles in which agglomerates are broken down and the particles are fully coated with cocoa butter (Minifie, 1989). The particle size of the solids in the chocolate matrix must be sufficiently small in order to minimise the grittiness of the chocolates. Indirectly, particle size also greatly influences the textural and rheological properties of chocolate (Do et al., 2007; Mongia and Ziegler, 2000). To be able to create chocolate with the desired particle size, the gap between the rolls which determines the pressure perceived by sugar and cocoa solids, should be well adjusted. Optimisation, involving the size of sugar particles and cocoa solids as well as the fat content of the mixture as variables, is required for this processing step. In some types of roll-refiners, the pressure between the rolls is automatically controlled and the temperature is also thermostatically adjusted. Thus, overheated rolls can be prevented, which can influence not only the physicochemical characteristic of mixture but also the roll distortion (Minifie, 1989).

Conching is usually carried out in a conche machine by agitating the chocolate under constant mixing and shearing action at a temperature higher than 50°C for several hours. Mixing and shearing involve the use of blades which have wedge-shaped ends attach to their rotors. At the early stage of conching, the wedge smears the powdery mixture along the conche wall. Once the chocolate has become liquid the rotor is reversed producing a higher shearing action within the mass (Beckett, 2009). Conching is a very time-and energy-consuming stage, but it is very crucial since it enables moisture reduction and fine-tuning of a desirable flavour profile. Aside from this, the textural and rheological characteristics of chocolate are also developed in this stage (Afoakwa, 2010; Beckett, 2009; Lucisano et al., 2006; Minifie, 1989). Conching time and temperature vary considerably, depending on the type of chocolate (Awua, 2002). For milk chocolate, conching temperature can be set only up to 60°C. Nevertheless, the conching temperature can be increased if a caramelised flavour produced through Maillard reaction is preferred (Beckett, 2009; Bolenz et al., 2003). For dark chocolate, conching temperature can be set up beyond 80°C (Afoakwa, 2010).

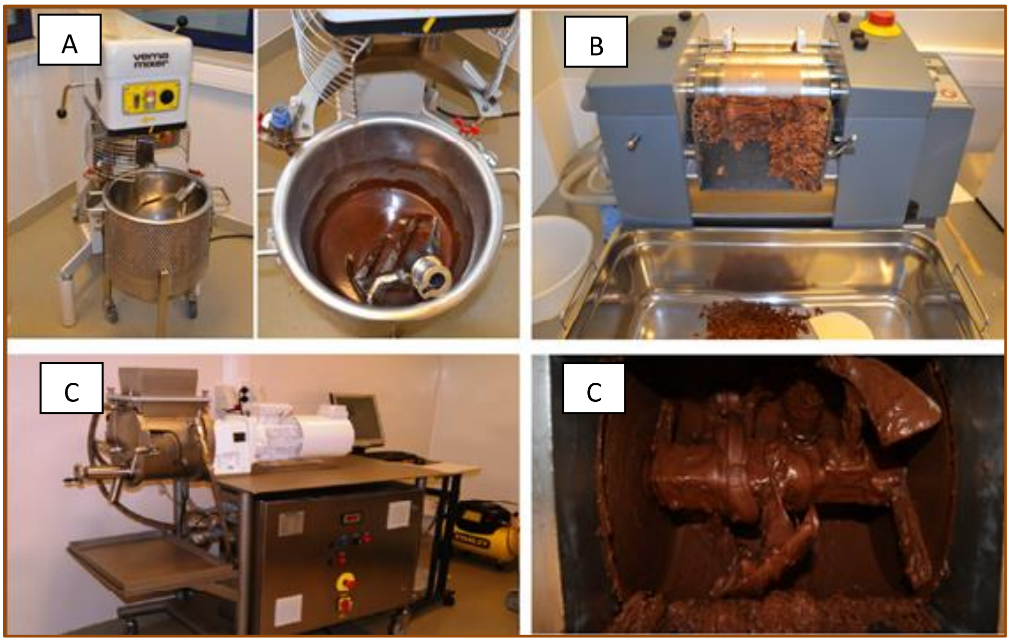


Figure 1.3. Typical machines utilised in conventional chocolate production system (Laboratory scale). (A) Vema mixer, (B) 3-roll refiner, (C) Buhler Elk'Olino conche

Conching can be divided into three phases, namely dry phase, pasty phase and liquid phase. During the dry and pasty phase, moisture present in the ingredients is evaporated together with the undesirable volatiles (development of aroma). At this moment, interaction between the solid particles and the cocoa butter takes place. When most of the solid particles are coated by the cocoa butter and the moisture is sufficiently low, the viscosity starts to decrease. The remainder of cocoa butter and emulsifier are added during the liquid phase which results in the solid particles being perfectly coated with cocoa butter. Sufficient time is necessary for the viscosity to reach its equilibrium (Afoakwa, 2010; Beckett, 2009; Bolenz and Manske, 2013; Minifie, 1989).

1.4.2.2 Alternative processing : ball-milling

A ball mill is a promising small-scale chocolate processing machine in which a milling and conching-like process are carried out at the same time (Figure 1.4) (Alamprese et al., 2007; Bolenz et al., 2014a), hence a compact production system is suitable for small-scale chocolate industry (Bolenz and Manske, 2013). In contrast to the conventional processing in which the chocolate production is sometimes carried out in an open container with high risk of contamination, the alternative processing using ball mill is conducted in a closed chamber (Bolenz et al., 2014a; Bolenz et al., 2014b). However, reducing moisture and undesired volatiles is a challenge (Bolenz et al., 2014a; Bolenz and Manske, 2013; Bolenz et al., 2014b; Lucisano et al., 2006). Apart from that, the chocolate produced using a ball mill requires a higher fat content to be able to flow during the entire milling process which is not conducive to the aforementioned moisture reduction (Bolenz and Manske, 2013; Bolenz et al., 2014b). To cope with the difficulties in evaporating moisture, pre-dried materials are mostly used, especially with regard to high-moisture material such as milk powder (Bolenz and Manske, 2013; Bolenz et al., 2014c; Lucisano et al., 2006). Another potential method is utilisation of a liquefier and/or a moisture removing device (Beckett, 2009; Lucisano et al., 2006). This device can be used to create a more homogeneous chocolate suspension after milling so that a good flowability of molten chocolate can be achieved. Nevertheless, only few studies have reported the use of a ball mill coupled with a liquefier device.

As the name implies, a ball mill consists of a cylinder/tank that contains numerous stainless steel balls and a rotating shaft with arms (Alamprese et al., 2007; Beckett, 2008). Refining process is conducted either by rotating the wall or the shaft, making the balls collide against each other. In principle, as the balls collide and rotate, the particles caught in between are pressed and broken down or pulled apart as a result of shear forces generated by the rotation action (Beckett, 2008; Minifie, 1989).

Figure 1.4. Typical ball mill used in the alternative processing (Laboratory scale)

1.4.3 From molten chocolate to chocolate bar

The last steps of chocolate bar production are tempering and moulding. Indeed, prior to the consumption, crystal maturation is needed in order to ensure that the chocolate has the desired polymorphic form which will result in a pleasant mouthfeel. In addition, it also determines the shelf-life of the chocolate.

1.4.3.1 Tempering

Tempering is a thermo-mechanical treatment of molten chocolate to create a sufficient number of homogeneously dispersed and highly stable fat crystals in the correct type and size. These crystals act as seeds for the crystal growth ensuring a compact crystalline structure during the subsequent cooling stage (Delbaere et al., 2016; Windhab, 2009). Properly tempered chocolate gives rise to chocolate with desired characteristics in terms of contraction (easily to be demoulded), gloss and snap as well as optimal stability against fat and/or sugar bloom under normal storage conditions (Delbaere et al., 2016; Minifie, 1989).

Due to its relatively simple triacylglycerol composition, cocoa butter crystallises in a number of polymorphic forms, with each polymorphic form exhibiting a specific melting point and crystal structure (Talbot, 2009). A mixed nomenclature, namely a Roman numeral one (Wille and Lutton, 1966) and a Greek letter (van Malssen et al., 1999), is used to differentiate those forms. Polymorphic form I / sub-α / γ; polymorphic form II / α; polymorphic form III / β'; polymorphic form IV / β'; polymorphic form V / βv ; and polymorphic form VI / β_{VI} has a melting temperature of 17.3 °C, 22.3 °C, 25.5 °C, 27.5 °C, 33.8 °C, and 36.3 °C, respectively. βv is the most preferable polymorphic form in chocolate due to its ability to give the highest surface gloss and hardness/snap, smooth melting and shelf-life characteristics (Windhab, 2009).

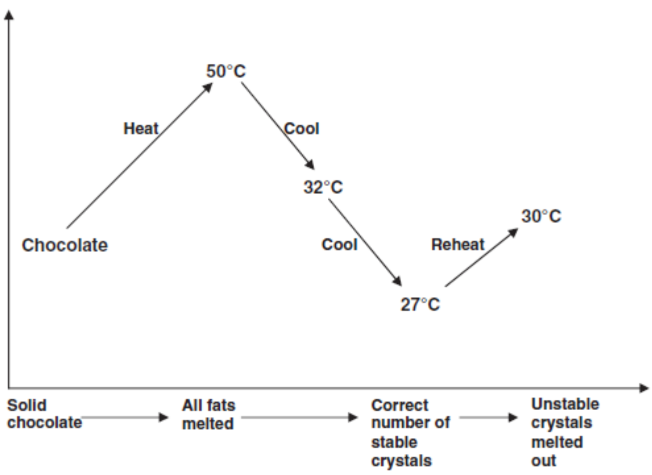


Figure 1.5. Sequence of processes during chocolate tempering (Afoakwa, 2010)

Conventionally, tempering is manually carried out on a marble table. This process requires skilled people and is suitable for small quantities. Aside from this, seeding techniques using seed crystals consisting of 30–95% β_{VI} polymorphs in addition to the β_{V} can also be used (Windhab, 2009). However, at industrial scale, automatic tempering machines are more frequently used (Afoakwa, 2010; Talbot, 2009). In the latter method, chocolate is subjected to a well-defined temperature program under shear. This process induces the formation of seed crystals from which the appropriate polymorphic is formed. This method has four key steps, namely melting to completion (at 40-50°C), cooling to point of crystallisation (at 32°C), crystallisation (at 27°C) and conversion of any unstable crystals (at 29–31°C) (Figure 1.5) (Afoakwa, 2010; Talbot, 1994).

1.4.3.2 Moulding and maturation

In chocolate bar production, molten chocolate can be moulded once the tempering process is done. To ensure that the chocolate has the correct polymorphic form, maturation of the cocoa butter crystals for a certain period of time is sometimes needed, particularly for chocolate with high level of cocoa butter (Windhab, 2009).

1.5 QUALITY PARAMETERS OF DARK CHOCOLATE

The acceptability of chocolate is determined by its quality attributes, such as fineness, melting profile, flow behaviour, texture, appearance and flavour. These attributes can be evaluated indirectly by investigating some parameters that directly influence these quality parameters, such as moisture content, particle size distribution, rheological behaviour, hardness, colour, melting profile, aroma profile and microstructure of chocolate.

1.5.1 Moisture content

The moisture content of chocolate should be maintained as low as possible since it highly affects the quality attributes of chocolate, mainly its rheological behaviour (Section 1.5.3) and textural properties, such as hardness (Section 1.5.4). Beckett (2008) estimated that approximately for every 0.3% extra moisture that remains in the chocolate after conching, an extra 1% cocoa butter should be added to obtain chocolate with good quality attributes. This phenomenon implies that in the chocolate manufacture, ingredients with very low moisture are imperative. Moreover, an optimal dry conching process is also needed so that the moisture can be evaporated as much as possible.

1.5.2 Particle size distribution

The particle size distribution (PSD) is a key factor which has both direct and indirect influence on the quality attributes of chocolate. Fineness of chocolate is directly influenced by particle size which can be felt during chocolate consumption. Thus, palate sensitivity should be considered in the optimisation of the size of solids particles. For instance, chocolate which has a particle size of ≥ 30 µm might be perceived as 'gritty or coarse' chocolate (Afoakwa, 2010; Do et al., 2007). On the other hand, the grittiness of the particles cannot be felt when the particle size ≤ 30 µm (Bolenz and Manske, 2013). Nevertheless, the size of the particles in chocolate are product specific. Thus, for instance, chocolate made for cookie/biscuit is allowed to be coarser than solid-eating chocolate because the texture of the cookie masks the grittiness of the chocolate (Ziegler and Hogg, 2009). With regard to the indirect influence, the PSD influences the rheological behaviour (Section 1.5.3), hardness

(Section 1.5.4) and aroma profile of chocolate, at least to some extent (Section 1.5.7) (Afoakwa et al., 2009a; Afoakwa et al., 2008e, f; Do et al., 2007).

In the chocolate suspension, the amount of solid particles from sugar and cocoa solids which occupy a certain volume of chocolate can be quantified using a so-called "particle volume fraction" (Equation 1.1). The higher the amount of solid particles in the chocolate, the higher the particle volume fraction is and, as a consequence, the more pronounced the particle-particle interactions in the suspension are. The particle volume fraction ($\emptyset$) reaches its maximum value ($\emptyset_m$) once the particles are located in a 3-dimensional contact throughout the suspension, thus the suspension loses its mobility (Do et al., 2007; Mongia and Ziegler, 2000; Servais et al., 2002).

$$\emptyset = \frac{V_{particles}}{V_{particles} + V_{medium}}$$

Equation 1.1 Particle volume fraction of chocolate suspension (Do et al., 2007).
$(V_{particles} = V_{sugar\ particles} + V_{cocoa\ solids};\ V_{medium} = V_{cocoa\ butter})$

The viscosity of chocolate suspension at a given particle volume fraction ($\emptyset$) can be reduced by increasing the maximum volume fraction ($\emptyset_m$). This can be done by mixing small particles with the larger ones (Do et al., 2007; Mongia and Ziegler, 2000; Servais et al., 2002). The small particles may fill the voids/gaps between the large ones, thus reducing the amount of cocoa butter needed to fill the voids. In this case, small particles act as a lubricant for the larger particles, thus decreasing the viscosity (Do et al., 2007; Servais et al., 2002). In addition, more "free" cocoa butter is then also available for the flow, which further reduces the viscosity. Theoretically, a significant increase of maximum particle volume fraction ($\emptyset_m$) occurs when the size of the small particles are 7 times smaller than the big ones.

The presence of big and small particle groups in the chocolate suspension can be recognised from its PSD shape. Chocolate which has a "uniform" particle size, tends to exhibit a unimodal particle size distribution curve, while chocolate which has more than one group of particle sizes exhibits a multimodal PSD. Mongia and Ziegler (2000) and Do et al. (2007) stated that the occurrence of a multimodal or generally broader PSD results in a higher maximum particle volume fraction ($\emptyset_m$).

1.5.3 Rheological behaviour

Rheological behaviour is one of the most important quality attributes of chocolates since it does affect not only the mouthfeel and consumer acceptance but also the handling properties of molten chocolate, such as mixing and pumping, as well as enrobing, shell formation and moulding (Afoakwa et al., 2007; Servais et al., 2004). Therefore, controlling the rheological properties of chocolate is imperative.

Molten chocolate exhibits a non-ideal plastic behaviour, more particularly shear-thinning behaviour which occurs as soon as the yield value has been exceeded (Afoakwa, 2010; Aidoo, 2015). To fit this behaviour, there are several models that are available, such as Herschel-Bulkley, Bingham and Casson model. Casson model is the most widely used model within the chocolate industry eventhough there are still a lot of discussion about that in the last two decades. In 2000, the results from an inter-laboratory study (Aeschlimann and Beckett, 2000) showed that the Casson model was limited in accuracy, especially at low shear rates (Afoakwa et al., 2009b; De Graef et al., 2011). Hence, an alternative measurement method to obtain more accurate data is needed.

From Casson model, Casson yield stress, which is defined as the stress required to start the flow, and Casson viscosity, which refers to the internal friction during the flow, are derived (Equation 1.2) (Afoakwa, 2010; Beckett, 2009; Mongia and Ziegler, 2000). In the chocolate production, Casson yield value and Casson viscosity are important for moulding (low shear condition) and pumping process (high shear condition), respectively (Mongia and Ziegler, 2000).

$$\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\eta_{CA}} \cdot \sqrt{\dot{\gamma}}$$

Equation 1.2 Casson model (τ : shear stress, τ_{CA}: Casson yield value, η_{CA}: Casson plastic viscosity, $\dot{\gamma}$: shear rate)

Another rheological behaviour parameter that can be obtained from the measurement is thixotropy. This value is useful to evaluate the presence of agglomeration in the chocolate suspension which can be obtained by determining the difference between the ramp up and the ramp down shear stress at 5 s^{-1} (Afoakwa, 2010). A well-conched chocolate has a low thixotropy value.

The rheological properties of chocolate are highly influenced by moisture, fat and lecithin content as well as PSD (Afoakwa, 2010; Beckett, 2009; Chevalley, 1994; Do et al., 2007; Mongia and Ziegler, 2000). The moisture in the chocolate suspension can hinder the flow and, thus, has to be coated with fat so that the chocolate will have better Casson yield value and Casson viscosity. In addition, fat also limits the occurrence of particle agglomeration which is induced by moisture, thus resulting in a lower thixotropy value. Furthermore, the amount of lecithin added also helps lower the Casson Yield value, Casson viscosity and thixotropy value to some extent. Thus, by adjusting the formulation, a good rheological behaviour can be maintained. Apart from the ingredient-related factors, rheological behaviour is also influenced by PSD. Chocolate with a smaller particle size exhibits a higher Casson yield value and Casson viscosity compared to chocolate with a bigger particle size. This phenomenon can be associated with the surface area of the particles. Chocolate with small particle size has higher surface area which can induce higher particle-particle interactions in the chocolate suspension (Afoakwa et al., 2008a; Do et al., 2007; Mongia and Ziegler, 2000). However, it is not the case if small particles are present together with big particles. This chocolate will exhibit multimodal PSD behaviour. In the multimodal PSD, small particles fill the voids between the big ones (see section 1.5.2 : particle size distribution) and replace the cocoa butter in the voids. Thus, more cocoa butter is available for the flow and, as a consequence, the viscosity is decreased (Do et al., 2007).

1.5.4 Hardness

The hardness of chocolate influences textural sensation and also typical sound (snap) produced during the consumption of the chocolate which affect the consumer preference. It is mainly influenced by moisture content, fat content, particle size distribution and degree of tempering (Afoakwa et al., 2008e, 2009c; Shah et al., 2010; Stortz and Marangoni, 2011). The presence of moisture in the chocolate matrix may create sugar networks which increase the hardness of chocolate (Stortz and Marangoni, 2011). Fat content and particle size distribution are two factors responsible for particle-particle interactions within the chocolate matrix. A lower amount of fat and a smaller particle size result in more particle-particle interactions, creating a harder chocolate (Afoakwa et al., 2008f). Another factor that influences the hardness of chocolate is the degree of tempering. Compared to the well-tempered chocolate which has an appropriate polymorphic form, under-tempered chocolate has a lower hardness (Shah et al., 2010), while over-tempered chocolate has a higher hardness (Afoakwa et al., 2008e)

After tempering which is followed by subsequent moulding and maturation, a structure of solid particles dispersed in solid cocoa butter matrix is formed. This structure relies on the chocolate formulation and processing condition applied. At the microstructural level, chocolate with less cocoa butter content is characterised by the presence of dense solid particles. By investigating the microstructure of chocolate using Scanning Electron Microscopy (SEM), some quality attributes of chocolate which are mainly influenced by particle-particle interaction can be estimated. A typical chocolate microstructure, visualised using SEM by Delbaere et al. (2016) can be seen in Figure 1.6. The microscopic image show that the solid particles are densely packed in a flaky cocoa butter medium. The sugar crystals can be recognised by their sharp-and-irregular shape and the cocoa particles by their rounded shape.

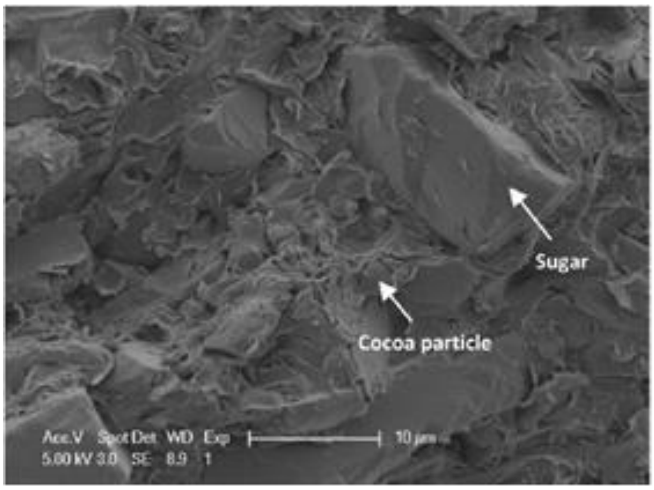


Figure 1.6. SEM images of a dark chocolate microstructure (Delbaere et al., 2016)

1.5.5 Appearance

The appearance of chocolate is highly determined by its glossiness, colour and roughness (Briones et al., 2006). Good quality chocolate has a glossy appearance with light to dark brown colour (Afoakwa, 2016). The glossiness is mainly influenced by the occurrence of fat and/or sugar bloom, while the colour, similar to the hardness, is influenced by the particle size and fat content which further influence the particle-particle interaction in the chocolate matrix. Chocolate which has more particle-particle interactions tends to be denser, scatters more light, and thus appears lighter and more saturated than chocolate with less particle-particle interactions (Afoakwa et al., 2008e, f). Aside from this, the colour of chocolate is also affected by the roughness of the chocolate surface. Briones et al. (2006) reported that chocolate with a smooth surface had a lighter colour than chocolate with a rough surface.

1.5.6 Melting profile

The melting profile of the cocoa butter crystals together with rheological and textural properties determine mouthfeel and flavour release (Afoakwa, 2010; Beckett, 2009). The intensity of the chocolate taste and flavour perceived changes as a function of time following its melting (Afoakwa et al., 2007; Ziegler and Hogg, 2009). The melting profile of cocoa butter is mainly determined by the degree of tempering, from which a certain polymorphic form is built. Thus by conducting this measurement, the chocolate hardness can also be examined (Delbaere et al., 2016; Minifie, 1989). More information about cocoa butter polymorphism and tempering process is described in Section 1.3.2 and Section 1.4.3.1.

1.5.7 Aroma profile of dark chocolate

The aroma profile of dark chocolate is mainly affected by the cocoa solids, the sugar state, and to some extent by the particle size distribution. The cocoa aroma and aroma precursors developed during postharvest handling and chocolate processing, including fermentation (Section 1.4.1.1), drying (Section 1.4.1.2), roasting (Section 1.4.1.4) and conching (Section 1.4.2.1) are proven to determine the final aroma profile of chocolate (Afoakwa, 2010, 2016; Aprotosoaie et al., 2016; Bonvehi, 2005; Counet et al., 2002b; Frauendorfer and Schieberle, 2008; Kongor et al., 2016). With regard to the influence of sugar state, Beckett (2008) stated that the presence of amorphous sugar resulted in a more intense chocolate flavour. This phenomenon was attributed to the ability of the amorphous state in absorbing the aroma volatiles during chocolate production. Aside from the aforementioned factors, Afoakwa et al. (2009a) also reported that dark chocolates with a finer particle size (18 and 25 µm) released more cocoa-chocolate-praline and caramel-like-sweet-honey notes than dark chocolates with larger particle size (35 and 50 µm). The increase in surface area with decreasing PS D(v,0.9) is suspected to facilitate the release of volatiles.

CHAPTER 2

SUCROSE REPLACEMENT :
AN INTRODUCTION TO PALM SAP SUGAR

CHAPTER 2
SUCROSE REPLACEMENT: AN INTRODUCTION TO PALM SAP SUGAR

2.1 SUCROSE AS THE MOST COMMON CHOCOLATE SWEETENER

Sucrose, the most common sugar used in chocolate production (Afoakwa, 2016; Beckett, 2009), is a disaccharide composed of the chemically linked monosaccharides glucose and fructose (Aidoo et al., 2013; Beckett, 2009; Eisenberg, 1955; Mathlouthi and Reiser, 1995). This link can be broken down through acid hydrolysis or enzymatic hydrolysis using an invertase enzyme (ß-d-fructofuranosidase) (Beckett, 2009). Sucrose is also frequently used as sweetener in baked foods, drinks, jams, jellies and preserves. It is industrially made from either sugar cane or sugar beet (Afoakwa, 2016; Asadi, 2006). To produce sucrose, purified juice extracted from the sugar cane or beet is concentrated and crystallised (Mathlouthi and Reiser, 1995). About 40% of the world's sucrose is made from sugar beet and 60% is made from sugar cane. Sugar beet is grown in moderately cold areas, while sugar cane is more suitable in tropical areas (Asadi, 2006; Mathlouthi and Reiser, 1995). In terms of yield, sugar beet and sugar cane have almost similar sucrose content, which is typically about 18% and 15% of sucrose, respectively (Asadi, 2006). In the market, the source of sucrose is normally not mentioned by producers due to its similarity. It is almost impossible to differentiate sucrose from sugar beet and sugar cane sold in the market, even for an expert (Asadi, 2006).

As a market practice, crystallised sucrose is graded based on its purity and crystal size. The purity of sucrose is generally higher than 99.9% and rarely below 99.7% (Beckett, 2009; Beckett et al., 2006) with water as the major impurity (Asadi, 2006; Mathlouthi and Reiser, 1995). The water content of good quality crystallised sucrose must not exceed 0.06%, while the presence of invert sugar (glucose and fructose) should be less than 0.04% (Beckett, 2009). The ash content should be less than 0.01% for refined sugar. Sucrose is used as standard reference in sensory tests to compare the sweetness of other sugars with 'sweetening power' or 'degree of sweetness' as terminology (Asadi, 2006; Beckett, 2009).

The crystal size of the sucrose varies depending on the commercial purposes. The following grading is generally used to classify sugar based on its size: coarse sugar (1-2.5 mm), medium fine sugar (0.6-1.0 mm), fine sugar (0.1-0.6 mm) and icing sugar (0.005-0.1 mm) (Asadi, 2006; Beckett, 2009). There is no standard regarding grain or particle size (Beckett, 2009). For instance, household consumers in the United States generally prefer to use fine sugar, while those in Europe prefer to use medium-size sugar, and those in Asia and Africa prefer to use coarse sugar (Asadi, 2006). At the industrial level, however, the baking industry prefers extra-fine sucrose because the mixing efficiency during processing improves. This results in a dough with a higher rising property and finely baked products with better quality (Asadi, 2006).

During production of crystalline sucrose, due to mechanical damage (Sun et al., 2012), an amorphous state can be generated, especially at the surfaces. The crystalline state is a solid state where molecules are well-arranged in a regular lattice, while the glassy amorphous state has disordered molecular arrangements (Figure 2.1) (Liu et al., 2006b). The presence of an amorphous state, to some extent, affects the physical property of crystalline sucrose. For instance, icing sucrose has a tendency to form lumps because the amorphous part, which is able to absorb moisture at higher rates and lower relative humidity, is present in freshly ground crystallised sucrose (Beckett, 2009). The presence of an amorphous state also influences the solubility of crystalline sucrose. Amorphous solids have a higher solubility than crystalline solids (Qian et al., 2010; Sun et al., 2012) since amorphous solids are less physically stable compared to the crystalline ones (Qian et al., 2010). Consequently, the presence of amorphous state in crystalline sucrose may increase the sucrose solubility.

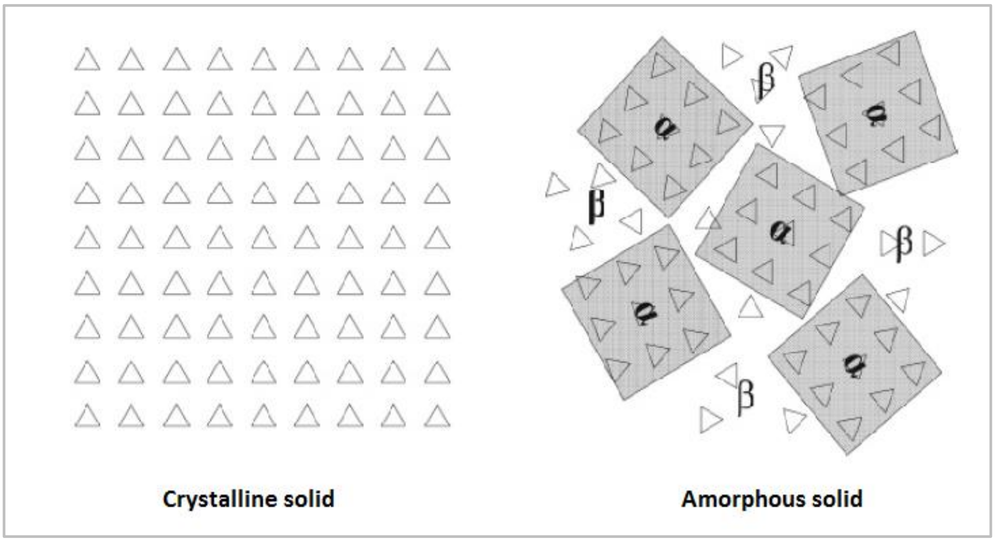


Figure 2.1. Difference in structure of crystalline and amorphous solid. In amorphous solid, the micro-heterogeneity is presented as the shaded high-density α regions and the non-shaded low-density ß regions (Liu et al., 2006b).

The amorphous state acts as a plasticiser for crystalline sucrose, thus decreasing the melting temperature, which is the temperature at which crystalline sucrose melts (Beckett et al., 2006; Ergun et al., 2010; Hadjikinova and Marudova, 2016). The amorphous fraction is inversely related to melting temperature. Thus, the higher the amount of amorphous state in the sucrose crystal, the lower the melting temperature (Bhandari and Hartel, 2002). The presence of amorphous state also lowers the enthalpy which is defined as the energy required to melt the crystal.

2.2 FUNCTIONALITY OF SUCROSE IN DARK CHOCOLATE

In the chocolate matrix, sucrose contributes to functional properties such as mouthfeel and can be used as texture modifier and bulking agent due to its high portion (Aidoo et al., 2013; Asadi, 2006; Cikrikci et al., 2016). Direct influence from the use of sucrose is related to the sweetness and fineness of chocolate. During chocolate production, sucrose is ground together with other ingredients. If the particle size is too large, grittiness will be perceptible (Afoakwa, 2010; Beckett, 2009). Indirect influences of sucrose are related to the rheological, textural and visual properties of chocolate. This can be linked to the particle-particle interactions between solids in the chocolate suspension (Afoakwa et al., 2007; Afoakwa et al., 2009d). In addition, Beckett (2008) stated that the presence of amorphous sucrose results in a more intense chocolate flavour. This phenomenon is attributed to the ability of amorphous state in absorbing the aroma volatiles during chocolate production.

2.3 ALTERNATIVE SWEETENERS FOR CHOCOLATE

Efforts to fully or partially replace sucrose as chocolate sweetener have been increasing mainly due to the food industry's search for a sweetener with low-calorie, low-glycemic index and high-sweetening power properties (Aidoo et al., 2013; Asadi, 2006; Furlán et al., 2017; Kroger et al., 2006). The most commonly used alternative sweeteners are low-digestible carbohydrates, bulk sweeteners and high potency sweeteners. To date, a number of research have been conducted to deal with this topic and most of them reported significant impact of alternative sweeteners not only on the quality attributes of chocolate, but also on the sensorial properties. This makes sucrose replacement very challenging.

2.2.1 Low-digestible carbohydrates

Low-digestible carbohydrates are incompletely absorbed in the small intestine but partly fermented by bacteria in the large intestine (Grabitske and Slavin, 2008, 2009). Inulin, polydextrose and resistant maltodextrin are the most common low-digestible carbohydrate used as bulking agents in chocolate manufacturing (Aidoo et al., 2013; Belscak-Cvitanovic et al., 2015; Farzanmehr and Abbasi, 2009; Rezende et al., 2015).

Inulin is widely found in nature, such as in leeks, onions, artichokes, garlic, wheat, and chicory roots (O'Donnell and Kearsley, 2012). It consists of fructose molecules linked together, ending with a glucose molecule (Figure 2.2). It behaves similar to bulking ingredients and can act as prebiotic (Franck, 2002; Shoaib et al., 2016). In the market, inulin is commonly available as hygroscopic powder (Franck, 2002; Srinameb et al., 2015). Polydextrose is a water-soluble polymer of glucose (Figure 2.3), which is produced by the condensation of glucose, sorbitol and small amounts of food grade acid (O'Donnell and Kearsley, 2012). Polydextrose provides the bulk and appropriate textural and mouthfeel qualities which is usually associated with sucrose properties (Aidoo et al., 2014; Burdock and Flamm, 1999). It is typically available as an amorphous powder, making it a hygroscopic material which can easily absorb moisture (Aidoo et al., 2013; Burdock and Flamm, 1999). Resistant maltodextrin is a product of starch hydrolysis which contains indigestible components. It is composed not only of α(1-4) and α(1-6) glucosidic bonds, but also (1-2) and (1-3) glucose linkages. In powder form, resistant maltodextrin is not as hygroscopic as polydextrose (Aidoo et al., 2013; O'Donnell and Kearsley, 2012).

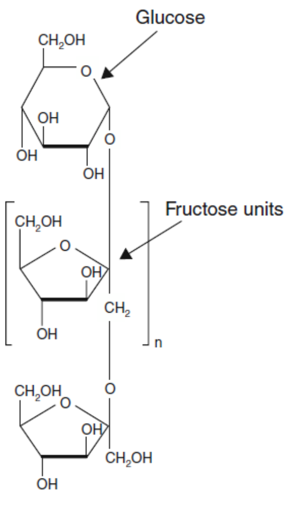


Figure 2.2. Chemical structure of inulin (O'Donnell and Kearsley, 2012)

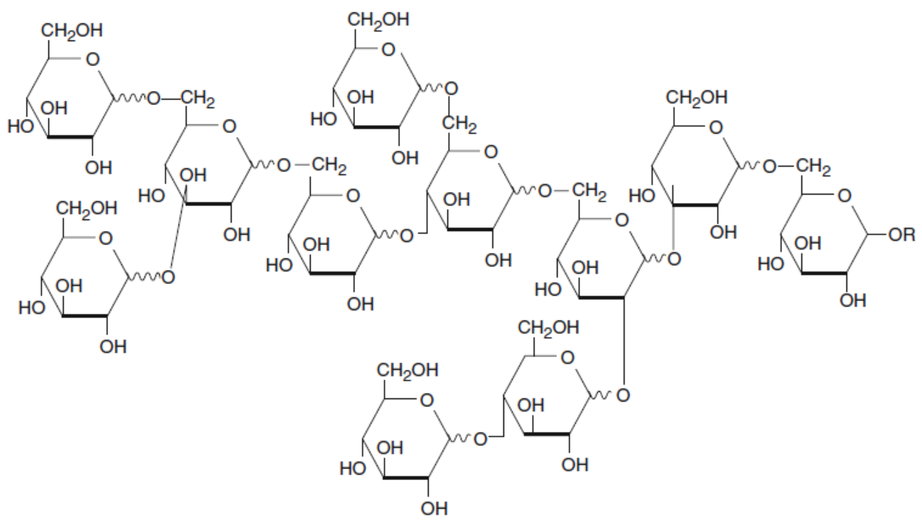
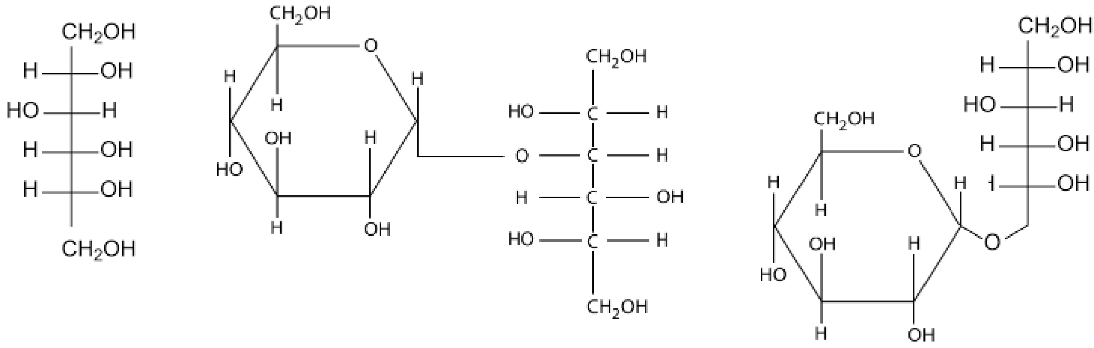


Figure 2.3. Chemical structure of polydextrose (O'Donnell and Kearsley, 2012)

2.2.2 Bulk Sweeteners

Bulk sweeteners are widely used in food products and are commonly referred to as “sugar replacers”. Unlike other alternative sweeteners earlier mentioned, bulk sweeteners can substitute to a high extent both the sweetness of sucrose and its physical bulking properties (Kroger et al., 2006). Bulk sweeteners usually replace sugar on a 1-to-1 basis (*i.e.*, 1 g of bulk sweetener replaces for 1 g of sucrose). Thus, they can provide comparable solid state to sucrose in chocolate suspension (Kroger et al., 2006).

The most common bulk sweeteners used in chocolate are sugar alcohols (polyols), such as sorbitol, maltitol and isomalt (Cikrikci et al., 2016; Konar et al., 2014; Nebesny and Żyżelewicz, 2005; Sokmen and Gunes, 2006). Polyols vary in sweetness from half to about as sweet as sucrose (Aidoo et al., 2013). Sorbitol is a hygroscopic bulk sweetener, which has a melting point of 97.2^{o}C. Its sweetening power is 60% of that of sucrose. Isomalt has only 50% of the sweetness of sucrose. Thus, in chocolate, it is normally used in combination with intense sweeteners. Isomalt is not hygroscopic and has a melting point between 145-150^{o}C. In contrast, maltitol is very hygroscopic with 85% sweetening power of sucrose. Due to its high sweetening power, it is usually not used in combination with intense sweeteners. This sugar is very hygroscopic with a melting point of 130-135^{o}C (Aidoo et al., 2013; O'Donnell and Kearsley, 2012). The chemical structures of sorbitol, maltitol and isomalt are shown in Figure 2.4.



(A) (B) (C)

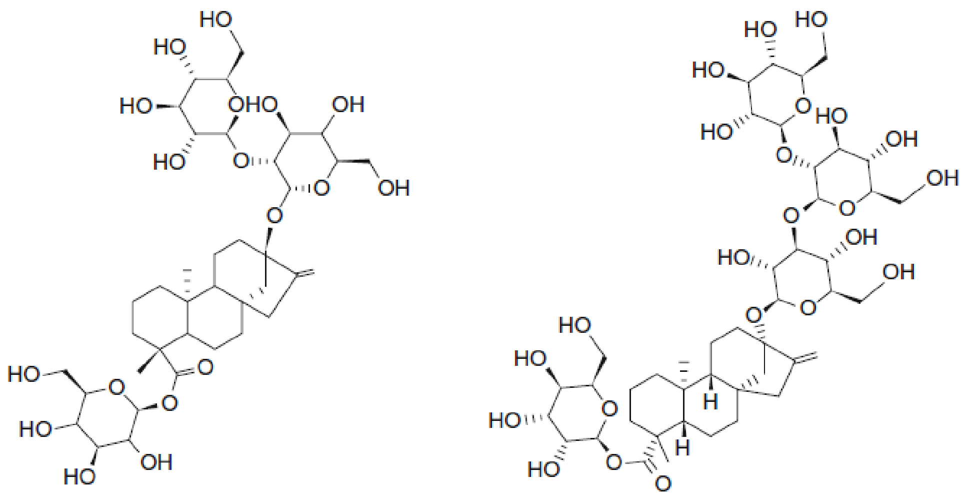
Figure 2.4. Chemical structures of (A) sorbitol, (B) maltitol, and (C) isomalt

Other bulk sweeteners that have potency to be used as chocolate sweetener are tagatose, trehalose and isomaltulose. The sweetening power of tagatose, trehalose and isomaltulose are approximately 90%, 45% and 50% of that of sucrose, respectively. Tagatose is a bulk sweetener with moderate higroscopycity and has melting point between 133-137^{o}C. Trehalose is not hygroscopic under relative humidity (RH) of 90% and has melting point of 97 ^{o}C (dihydrate) or 210.5^{o}C (anhydride). Isomaltulose is a bulk sweetener which has low hygroscopicity with melting point between 123-124 ^{o}C (O'Donnell and Kearsley, 2012).

2.2.3 High-potency sweeteners (high-intensity sweeteners)

High potency sweeteners consist of substances with a very intense sweetness that can be used in small amounts to replace the sweetness of a much larger amount of sugar (Kroger et al., 2006). It has degree of sweetness hundreds to thousand times higher than that of sucrose (Aidoo et al., 2013). Due to the absence of bulking property, the use of high potency sweetener faces a serious challenge in chocolate application. Therefore, it is combined with bulk sweeteners and/or low-digestible carbohydrates (Cikrikci et al., 2016).

The most common high-potency sweeteners used in chocolate are stevia and thaumatin (Aidoo et al., 2014; Cikrikci et al., 2016; Melo et al., 2007; Shah et al., 2010). Stevia which contains stevioside and rebaudioside A (Figure 2.5) as the main sweetening compounds is a natural sweetener with sweetening power of approximately 300 times as sweet as sucrose by weight (Cikrikci et al., 2016; Goyal and Goyal, 2010). Thaumatin is an intense, sweet-tasting protein which has a sweetening power 100,000 times sweeter than sucrose on a molar basis and 3000 times on a weight basis (Aidoo et al., 2014; Aidoo et al., 2013). Thaumatin comprises a mixture of at least five sweet forms with two major components, namely thaumatin I (60%) and thaumatin II (20%), and three minor components (thaumatin a, b, and c) (Masuda et al., 2011). To the best our knowledge, the chemical structures of thaumatin components are still not available in literature.



(A) (B)

Figure 2.5. Chemical structure of (A) stevioside, (B) rebaudioside A

Apart from stevia and thaumatin, there are some other types of high-potency sweeteners that can be used in chocolate, such as sucralose, saccharin, aspartame, acesulfame-K and neothame. The sweetening power of sucralose, saccharin, aspartame, acesulfame-K and neothame are approximately 300, 300, 180, 200 and 7000 times sweeter than that of sucrose, respectively (Kroger et al., 2006). To date, there are only very limited scientific studies on the use of these sweeteners on chocolate (Cikrikci et al., 2016; Furlán et al., 2017).

2.4 FUNCTIONALITY OF ALTERNATIVE SWEETENERS IN CHOCOLATE

A comprehensive map of the influence of alternative sweeteners on chocolate cannot be drawn yet because studies that focused on this topic used different formulations, emulsifier concentrations and chocolate types. However, it is clear that they surely affect the rheological behaviour (Aidoo et al., 2017; Farzanmehr and Abbasi, 2009; Konar, 2013; Nebesny and Żyżelewicz, 2005; Shah et al., 2010; Sokmen and Gunes, 2006), textural properties (Aidoo, 2015; Belscak-Cvitanovic et al., 2015; Farzanmehr and Abbasi, 2009; Konar, 2013) and colour of chocolate (Aidoo, 2015; Konar, 2013). A limited number of studies also investigated the impact on the melting properties (Aidoo et al., 2017; Shah et al., 2010) and fineness of chocolate (Belscak-Cvitanovic et al., 2015). To the best of our knowledge, there is no research about the influence on alternative sweetener on the aroma profile of chocolate. Due to the significant impact of alternative sweeteners on quality attribute of chocolate, partial replacement of sucrose and processing variables adjustment are frequently conducted to achieve the desired chocolate characteristics that are close to those of sucrose-sweetened chocolate.

Apart from the influence of alternative sweeteners on the quality attributes of chocolates, the use of alternative sweetener also results in different sensorial properties. This can be a disadvantage of alternative sweetener-sweetened chocolate. For instance, the result of a study conducted by Cikrikci et al. (2016) showed that stevia created chocolate with dark taste of stevia, which was not preferred by the panellists. Thus, fully replacement of sucrose with stevia is not a good option. Instead, partial replacement can be the best choice. Another, disadvantage of using alternative sweeteners, more particularly sugar alcohols is the fact that they are incompletely digested. Undigested sugar alcohols have an osmotic effect which pull water into the intestine. This lead to softer stools and/or even to diarrhea. Moreover, fermentation of undigested carbohydrates, such as polydextrose and inulin, by bacteria results in flatulence (Kroger et al., 2006; Lee and Storey, 1999; Zumbe et al., 2001). Aside from this, sugar alcohols and intense sweeteners are more expensive than sucrose which, to some extent, may also influence the consumer's preference (Zumbe et al., 2001)

2.5 PALM SAP SUGAR

2.5.1 Introduction

Palm sap sugar is a natural sweetener made from sap/nectar collected from the flowers of several species of palm tree, such as sugar palm (*Arenga pinnata*), palmyra palm (*Borassus flabellifer*), nipa palm (*Nypa fruticans Wurmb*) and coconut palm (*Cocos nucifera*) (Apriyantono et al., 2002; Arcieri, 2014; Ho et al., 2007, 2008b; Phaichamnan et al., 2010a; Purnomo, 2007; Tomomatsu et al., 1996). This sugar has been used as a traditional and an alternative sweetener in the South-East and South Asian regions, such as Indonesia, Philippines, Thailand, Malaysia and India. In these regions, the above-mentioned species of palm tree are highly grown (Ministry of Industry of The Republic of Indonesia, 2010), with Indonesia and the Philippines as the largest palm sap sugar producers in the world (Broberg, 2014).

Palm sap sugar is mainly utilised in sweet soy sauce, beverages, desserts, and various numbers of traditional foods. Its use is essential for the taste, colour and aroma development of the drinks and foods (Arcieri, 2014; Ho et al., 2007; Hori and Purboyo, 1991; Purnomo, 2007; Tomomatsu et al., 1996). Based on these functionalities, palm sap sugar can be considered as a potential natural sweetener (Suwansri et al. (2009). In a work carried out by Apriyantono et al. (1996), the utilisation of palm sugar as soybean sauce sweetener was reported to highly influence the flavour of soy sauce due to the presence of more than 70 volatiles.

A number of studies about palm sap sugar have been conducted, mostly related to its processing and its quality profile (proximate content, aroma profile, reducing sugars, protein, minerals and antioxidant activity) (Ho et al., 2006a; Ho et al., 2008b; Naknean et al., 2013; Phaichamnan et al., 2010a; Purnomo, 2007; Rao et al., 2009; Thumthanaruk et al., 2016; Tomomatsu et al., 1996). However, research on its functionality in products, such as in cherry puree (Nowicka and Wojdyło, 2016) and in soybean sauce (Apriyantono et al., 1996) is very limited. To the best our knowledge, the incorporation of palm sap sugar in confectionery products has not been scientifically studied yet.

2.5.2 Composition of sap as raw material for palm sap sugar production

Fresh sap / nectar of the palm tree's flower contains about 80% water, 10-15% total sugars comprised mainly of sucrose, some amount of reducing sugars, amino acids and minerals and vitamins (Ho et al., 2007; Philippine Coconut Authority, 2016; Purnomo, 2007; Rao et al., 2009; Tomomatsu et al., 1996; Xia et al., 2011). The composition of these substances varies depending on the variety of palm tree, stage of maturity of the inflorescence, climatic condition as well as soil fertility (Purnomo, 2007).

The rich, nutritious components make the sap/nectar vulnerable to spontaneous fermentation even during the process of harvesting, resulting in variable compositions. During this process, sucrose can be naturally converted to glucose and fructose by micro-organisms (Vidanapathirana et al., 1983). The availability of a high amount of reducing sugars such as glucose and fructose together with the presence of amino acids, as consequence, will result in a higher degree of Maillard reaction during the evaporation step under heating of the sugar production (Ho et al., 2008b; Purnomo, 2007). There are 16 kinds of amino acids present in fresh palm sap (Xia et al. 2011). As cited by Purnomo (2007), Fernandez (1983) observed that glutamic acid, threonine, aspartic acid and serine are the major amino acids present in fresh sap, while proline, methionine, tryptophan and histidine are present in minor amounts. Fresh palm sap also contains vitamin C and vitamin B complexes. Aside from this, phenolic compounds are also present. Together with vitamin C, phenolic compounds are important antioxidants (Xia et al., 2011). In addition, calcium, phosphorus and iron are present (Philippine Coconut Authority, 2016)

The major volatile compounds present in palm sap/nectar can be classified as esters, aromatic hydrocarbons, aliphatic ketones, alcohols, acids and heterocyclic compounds (Borse et al., 2007). This is in agreement with the work carried out by Purnomo (2007) in which it was reported that 2-butanol and acetic acid were the major volatile components identified in the fresh sap. During palm sugar production, the concentration of some of the volatile components decreased and in some cases they were not detectable in the final product (Purnomo, 2007).

2.5.3 Factors influencing the quality of sap

Palm sap sugar processing starts at harvesting/tapping the sap/nectar. This process can be done by cutting the flowers, followed by collecting the sap using usually a container made of bamboo (Broberg, 2014). Sap is highly susceptible to spontaneous fermentation which very rapidly occurs under sunlight. Aside from this, the contamination of the sap may occur during harvesting because the tapping process is conducted in an open-condition. These lead to changes in physical and chemical properties of the sap, including the level of acids (Phaichamnan et al., 2010a), reducing sugars (Samarajeewa and Wijeratna, 1983) and amino acids (Xia et al., 2011). Therefore, the quality profile of the sugar produced by farmers varies (Atputharajah et al., 1986; Borse et al., 2007; Samarajeewa and Wijeratna, 1983; Tomomatsu et al., 1996).

The composition of the sap/nectar, which influences the final product, is also affected by the timing and duration of the sap tapping (Borse et al., 2007). In his work, Purnomo (2007) also reported that sap collected in the morning and in the afternoon has slight compositional differences. Apart from that, the palm tree variety, stage of maturity of the inflorescence, climatic condition and soil fertility determine the quality of the sap/nectar (Borse et al., 2007; Hori et al., 2001; Purnomo, 2007).

2.5.4 Processing of palm sap sugar

In the market, palm sap sugar can be found as syrup, moulded sugar or powdered (coarse) sugar (Figure 2.6) (Broberg, 2014). The first type is made by boiling the sap/nectar until the sugar syrup reaches a certain degree brix (65-75^{o}Brix) (Naknean and Meenune, 2011b; Phaichamnan et al., 2010a; Purnomo, 2007). The palm sap syrup is usually stored in glass/plastic container. The second type is produced by boiling the sap/nectar until a very viscous sugar is formed. Afterwards, this sugar is traditionally moulded using coconut shell or bamboo moulds to form solid sugar (Ho et al., 2007, 2008b). To create the third type, further heating/boiling under agitation is needed, until the supersaturation point is reached and sugar crystals are formed. In the latter process, grinding is applied to obtain powdered sugar with uniform and specific sizes, followed by a drying process to further evaporate moisture left in the sugar.

Figure 2.6. Types of palm sap sugar sold in Indonesian market. (A) palm sap sugar syrup, (B) moulded palm sap sugar, (C) powdered (coarse) palm sap sugar

Prior to the processing, the sap/nectar is filtered to remove dirt/contaminants. It is then heated for several hours at about 100^{o}C-150^{o}C (Apriyantono et al., 2002; Ho et al., 2008a; Srikaeo and Thongta, 2015) until the desired form is reached. Essentially, palm sap sugar's form relies on the level of the moisture present in the sugar (Broberg, 2014), which is determined by the heating time and temperature. The moisture content has an inverse correlation with the processing duration. Higher moisture evaporation requires longer processing. Due to the different techniques applied in palm sap sugar production by farmers which include temperature and time, the characteristics of palm sap sugar in the market tend to vary (Phaichamnan et al., 2010b).

During palm sap sugar production, two major reactions are responsible for the flavour and colour development, namely Maillard reaction and caramelisation. The former reaction requires reducing sugar and amino acids as precursor (Apriyantono et al., 2002; Ho et al., 2008b; Naknean et al., 2009b), while the latter reaction requires high temperature (>120 ^{o}C) to take place (Kroh, 1994). Therefore, in order to minimise the prevalence of these reactions, a vacuum evaporator which enables the production at lower temperature and shorter time can be utilised (Naknean et al., 2013). By using this method, sucrose inversion can be reduced, thus, lowering the amount of glucose and fructose present in palm sap sugar.

2.5.5 Composition of palm sap sugar

Sugar is the primary substance in caramelisation reaction during heating. High temperature and long heating time will accelerate hydrolysis reaction of sucrose to glucose and sucrose (reducing sugars) (Figure 2.7) (Amin et al., 2010; Kroh, 1994; Phaichamnan et al., 2010a). Additional reducing sugars produced can then interact with amino acids via Maillard reaction, creating a distinctive aroma profile and a darker colour. Therefore, the amount of sucrose and reducing sugars in the final product depends on the form/type of the palm sap sugar. For instance, palm sap sugar syrup, which needs a shorter duration of production, has a higher amount of sucrose and reducing sugars compared to those of moulded or powdered sugar. This phenomenon is in agreement with the study carried out by Apriyantono et al. (2002) in which they reported that the amount of sucrose, reducing sugars, and amino acids decreased as the heating time was increased.

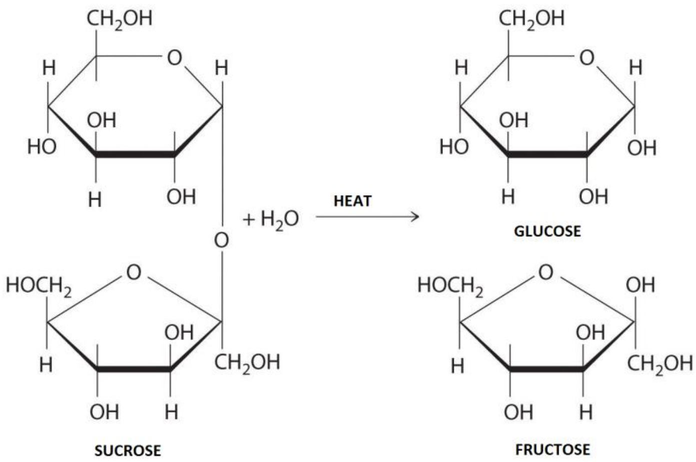


Figure 2.7. Hydrolysis reaction of sucrose during palm sap sugar production

Varying level of sucrose and reducing sugars in palm sap sugar were reported by some researchers due to differences in the raw materials and processing techniques used by farmers. Phaichamnan et al. (2010a) reported that total sugars of palm sap sugar syrup (59.0-73.1^{o}Brix) varied from 23.8 to 71.9%, while reducing sugars varied from 3.5 to 23.9%. Naknean and Meenune (2011b) informed that the main sugar found in palm sugar syrup (63.0-72.6^{o}Brix) was sucrose, found in the range of 59.2 to 84.4%, while the content of glucose and fructose was in the range of 4.0% to 24.1% and 4.4% to 23.6%, respectively. Arcieri (2014) stated that coconut sugar contained sucrose in the range of 70 to 79% as well as glucose and fructose, constituting 3 to 9% each. Purnomo (1992) reported that some palm sap sugar samples (75^{o}Brix) studied in his work contained 70.5 - 79.0% sucrose, 3.0% - 9.0% glucose and 2.9% - 9.0% fructose.

As amino acids are also involved in the Maillard reaction, their amounts in palm sap sugar tend to decrease as the processing time increases (Apriyantono et al., 2002; Ho et al., 2008a; Naknean et al., 2013). In the study of Ho et al. (2008b), it was reported that 15 types of amino acids were present in palm sap sugar with asparagine, glutamine and arginine as the major amino acids. Their concentrations were very high (>1 g/100 g) at the beginning, but significantly decreased after 120 min processing.

Apart from the aforementioned components, palm sap sugar also contains relatively high moisture content, minerals and vitamins. The Philippine Coconut Authority (2016) reported that coconut sugar contains phosphorus, potassium, calcium, magnesium, and iron. Arcieri (2014) stated that coconut sugar has an 18 times higher potassium content, 30 times higher phosphorus content and over 10 times higher zinc content than those of brown sugar (unrefined cane sugar). With regard to the iron content, its presence in palm sap sugar may have originated from palm sap and/or from iron utensils during concentrating of the palm sap (Hori and Purboyo, 1991). Moreover, palm sap sugar contains Vitamin B1, B2, B3, and B6. (Arcieri, 2014; Philippine Coconut Authority, 2016). Moisture content differs according to the type of palm sap sugar. Powdered sugar, which undergoes the longest processing duration, in contrast to palm sugar syrup, evaporates more moisture resulting in a lower moisture content. Apriyantono et al. (2002) reported that after 90 min of heating, the moisture content of palm sap sugar in his study was 6.95%. while in the work carried out by (Purnomo, 1992), 10.8-13.5% of moisture was obtained. Based on Indonesian National Standard regulation (BSN, 1995), moulded and powdered palm sugar should contain moisture maximum 10% and 3%, respectively.

2.5.6 Aroma profile and colour of palm sap sugar

The occurrence of Maillard reaction and caramelisation during palm sap sugar processing gives rise to a darker colour and more intense caramel flavour (Ho et al., 2006a; Ho et al., 2007, 2008b; Phaichamnan et al., 2010a). With regard to the aroma profile, similar to the aroma in most thermally processed foods, the N-, O- and S- heterocyclic compounds are present in major amounts (Wan Aida et al., 2008)

Apriyantono et al. (1996) identified 27 volatile compounds in coconut sugar whereby the major volatiles were dodecanoic acid (floral), acetic acid (sour), 2-undecanone (floral/fruity), decanoic acid (rancid), 2-nonanone (floral/fruity) and 2-furfural (sweet). In addition, regardless of the odour threshold value, 2-methyl pyrazine (roasted/caramel), 2,5-dimethyl pyrazine (roasted/caramel), 2,6-dimethyl pyrazine (roasted/caramel) and 2-acetyl pyrrole (nutty) were also found in smaller concentrations. On the other hand, Ho et al. (2006a) reported that 36 volatiles were observed in palm sugar which included 14 pyrazines, 5 furans, 2 furanones, 1 pyran, 6 aromatics, 4 acids, 2 aldehydes and 2 ketones. In another work, Ho et al. (2007) identified 30 aroma volatiles that consisted of 17 pyrazines, 7 furans, 4 aldehydes and 2 ketones. The difference on the identified aroma volatiles is mainly due to the different composition of the fresh sap and/or the difference of processing methods. In addition, the difference of the measurement methods used (type of instrument, column, preconditioning temperature, sample preparation *etc*) may also contribute to these results.

2.5.7 Factors influencing the quality of palm sap sugar

As previously mentioned, raw material and processing method have a direct influence on the quality of palm sap sugar. Due to these factors, palm sap sugar can have various physicochemical characteristics (Phaichamnan et al., 2010a; Purnomo, 2007; Tomomatsu et al., 1996). In addition, the form of palm sap sugar and storage also affect palm sugar quality to some extent. Palm sap sugar syrup, unlike powdered sugar, is prone to deterioration because of its high moisture content (Naknean and Meenune, 2011b). During storage, Maillard reaction can occur at a low extent, thus affecting the physicochemical properties of palm sap sugar (Naknean et al., 2013)

2.5.8 POTENCY OF PALM SAP SUGAR AS CHOCOLATE SWEETENER

The use of palm sap sugar as an alternative sweetener for chocolate could be interesting because of several reasons. Firstly, palm sap sugar is highly produced in Southeast Asia, *e.g.* Indonesia, Malaysia, Thailand, Philippines, where cacao is also (highly) produced. Thus, the valorisation of palm sap sugar may not only attract economic benefits but may also reduce the dependency on pure sucrose produced from sugar cane (*Saccharum officinarum*) in the countries within this region. In terms of price, eventhough the price of palm sap sugar in other continents is more expensive than that of sucrose, the price of the palm sap sugar in

Southeast Asia region is relatively similar to the price of sucrose made from sugar cane. Hence, palm sap sugar will be able to commercially compete against sucrose. Secondly, due to its sugar component and aroma profile, its utilisation may create distinctive and unique chocolate characteristics as commonly observed in the foods sweetened with palm sap sugar, for instance sweet soy sauce (Apriyantono et al., 1996). Thirdly, one type of palm sap sugar, namely coconut sugar, has been reported to have a health benefit due to its lower glycemic index (35–42) (Trinidad et al., 2010; Waldrop and Ross, 2014). Palm sap sugar utilisation in bread also resulted in lower glycemic index (GI) than that of bread sweetened with cane sugar (Srikaeo and Thongta, 2015). Apart from the GI value, palm sap sugar contains 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) (Ho et al., 2006a). DDMP is an anti-oxidative compound (Cechovska et al., 2011; Yu et al., 2013b) which also has potency to reduce the risk for colon cancer (Ban et al., 2007). In addition, as it has been mentioned before, palm sap sugar contains also antioxidants, vitamins, and minerals (Amin et al., 2010; Arcieri, 2014; Naknean and Meenune, 2011b; Philippine Coconut Authority, 2016). Therefore, the utilisation of palm sap sugar may also offer potential health benefits for chocolate consumers (Treccase et al., 2011).

CHAPTER 3

PHYSICOCHEMICAL PROPERTIES OF PALM SAP SUGARS FROM INDONESIAN ORIGIN

This chapter is prepared for publication :
Saputro, A.D., Van de Walle, D., Dewettinck, K.
Physicochemical properties of coarse palm sap sugars from Indonesian origin

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3.1 ABSTRACT

Palm sap sugar is an alternative chocolate sweetener, in lieu of sucrose, which is used due to its potential of creating chocolate with unique characteristics. Of the three different types of palm sap sugars (palm sap syrup, moulded sugar and coarse palm sap sugar), coarse palm sap sugar is the most suitable to be utilised in chocolate manufacture due to its relatively low moisture content. This research aimed to investigate the physicochemical properties of several coarse palm sap sugars and evaluate their potency as chocolate sweetener. In this study, coarse coconut sugars (CCS1, CCS2) and coarse palm sugars (CPS1, CPS2, and CPS3) of Indonesian origin were studied, while coarse pure sucrose was used as reference. The results showed that the moisture content of palm sap sugars was higher than that of sucrose. While proteins, reducing sugars and ash were only present in the palm sap sugars. Maillard reaction and caramelisation which occur during sugar production were responsible for the dark colour of palm sap sugars. Due to the presence of moisture, protein, reducing sugars, ash as impurities, the melting and glass transition temperature of palm sap sugars were lower than those of sucrose. Moreover, particle densities of palm sap sugars were lower and particle sizes of palm sap sugars were less uniform compared to those of sucrose. The presence of relatively high moisture and reducing sugars as hygroscopic material may be responsible for the presence of layers sticking to the surface of palm sap sugar crystals, visualised through scanning electron microscopy. The variation on the physicochemical properties of palm sap sugars can be attributed to the raw materials and processing methods used by the sugar producers. Therefore, the use of palm sap sugar in chocolate manufacture may affect the chocolate characteristics. Nevertheless, the extent of the influence of palm sap sugar on the chocolate characteristics should be investigated further.

3.2 INTRODUCTION

Palm sap sugar is an alternative sweetener that has the potential to be incorporated in chocolate manufacture as a substitute for sucrose. This sugar which is commonly used in various numbers of traditional foods in South-East and South Asian regions plays an important role in colour and flavour development of food products (Arcieri, 2014; Ho et al., 2007; Hori and Purboyo, 1991; Purnomo, 2007; Tomomatsu et al., 1996). Therefore, the use of palm sap sugar as chocolate sweetener may be able to create unique chocolate characteristics. Moreover, palm sap sugar is also claimed to have health benefits due to its low glycemic index, antioxidants, vitamin and mineral contents (Arcieri, 2014; Philippine Coconut Authority, 2016; Trinidad et al., 2010; Waldrop and Ross, 2014). It is abundantly produced in coconut-planting regions, mainly in Indonesia and the Philippines, where cocoa trees are also grown (Ministry of Industry of The Republic of Indonesia, 2010). Thus, its valorisation in this aspect may also attract economic benefits. Nevertheless, to ensure that the utilisation of palm sugar can yield chocolates with good quality characteristics, knowledge about the physicochemical properties of this sugar should be obtained.

The physicochemical characteristics of palm sap sugars, discussed comprehensively in Chapter 2, are highly affected by their raw materials and processing techniques (Phaichamnan et al., 2010a; Purnomo, 2007). Aside from this, the form of the sugars (syrup, solid, coarse/powder) also determines their properties. Several studies have been conducted by researchers which mainly focused on the production and chemical characterisation of palm sugar syrup and moulded palm sugar (Apriyantono et al., 2002; Ho et al., 2008b; Naknean et al., 2009b; Naknean et al., 2013; Phaichamnan et al., 2010a). To the best of our knowledge, the study on the physicochemical characteristics of coarse palm sap sugar has not been performed yet. Coarse palm sugar, due to its lower moisture content than the other forms, is undoubtedly the most appropriate form as chocolate sweetener. In addition, coarse palm sugar provides bulking property which is necessary as sucrose replacer (Aidoo et al., 2013; Kroger et al., 2006).

This research aimed to study the physicochemical properties of several coarse palm sap sugars and evaluate their applicability in chocolate manufacturing. Physicochemical analyses, such as levels of moisture, fat, proteins and reducing sugars, particle density, particle size distribution and thermal behaviour were carried out since they were expected to affect the quality characteristics of chocolate. Colour and microstructural visualisation were also conducted to support the results of the aforementioned analyses.

3.3 MATERIALS AND METHODS

3.3.1 Raw materials

Two kinds of palm sap sugars, namely coarse coconut sugar (CCS1, CCS2) and coarse palm sugar (CPS1, CPS2, and CPS3) were purchased from Sari Nira Nusantara CV. (Yogyakarta, Indonesia). As reference, coarse pure sucrose (CS) were obtained from Tiense Suikerraffinaderij (Tienen, Belgium).

3.3.2 Analytical Methods

3.3.2.1 Moisture content

The moisture content of the sugars was measured by Karl-Fisher titration method using a 719 Titrino device (Metrohm, Switzerland). Hydranal titrant 5 (Riedel de Haen, 34801) and hydranal solvent (Riedel de Haen, 34800) were used. About 1 g of sugar was used for each analysis. Measurements were done in triplicate.

3.3.2.2 Fat content

The fat content of the sugars was determined using Weibull method. Approximately 5 g of sugar was put into a 250 ml beaker. HCl (25 %) was then added and boiled for 15 min. Afterwards, the sample solution was filtered using filter paper and washed with hot water. The filter paper was then dried and placed into Soxhlet apparatus and was subsequently extracted with about 200 ml petroleum ether for 4 hours. The solvent was then evaporated to precipitate the fat. Measurements were done in triplicate.

3.3.2.3 Protein content

The protein content of the sugars was determined using Kjeldahl method and the result was calculated using a conversion factor of 6.25. About 0.5 g of sugar was digested by heating 10 ml concentrated H_2SO_4. Catalysts such as $CuSO_4$ and K_2SO_4 were added to the sugar prior to digestion. The digestion was finished when a bright green colour appeared. Afterwards, samples were placed in a distillation machine. The digest was subsequently distilled to release the ammonia. In the last step, the distillate was titrated using 0.05 N HCl. Measurements were done in triplicate.

3.3.2.4 Ash content

The ash content of the sugars was determined using a muffle oven. About 5 g of sugar placed in a crucible was heated on a heating plate to allow full carbonisation. Afterwards, the sample was put in a muffle oven for at least 4 hours at 500^{o}C. The residue left in the crucible was the ash measured. Measurements were done in triplicate.

3.3.2.5 Sucrose and reducing sugars content

Sucrose and reducing sugars content were determined by gas chromatographic analysis, following the method described by De Wilde et al. (2005), after an aqueous extraction. Prior to the extraction, phenyl-ß-D-glucopyranoside (6 mg/mL) was added as an internal standard. After 30 mins of incubation at 60°C, Carrez I (5 ml) and Carrez II (5 ml) were added. This solution was then filtered followed by drying of 1 mL of the filtered solution under nitrogen. The residue was firstly derivatised by adding oximation reagents (100 µl), secondly by adding hexamethyldisilizane (100 µl) and trifluoraaceticacid (10 µl).

A Varian 3380 gas chromatograph equipped with a flame-ionization detector (Varian Instrument Group, Walnut Creek, CA) was used for sugar separation. The parameters used were as follows: stationary phase: (5%-phenyl)-methylpolysiloxane, mobile phase: He at 1 mL/min, injector temperature: 250 °C; detector temperature: 340 °C; injection volume: 1 µL; temperature program: 180 °C for 1 min, ramp at a rate 15 °C/min to 290 °C. Measurements were done in triplicate.

3.3.2.6 Solubility

The solubility test was conducted at room temperature by quantifying the amount of sugar that can be completely dissolved in 40 ml distilled water. The sugar was added into distilled water and was subsequently agitated using automatic stirrer. Once the sugar solution reached its supersaturation point which is indicated by the inability of water in further dissolving the sugar, the test was stopped. Measurements were done in triplicate.

3.3.2.7 Particle density and bulk density

The particle density was measured using a pycnometer. About 10 g of sugar was put into the pycnometer. Afterwards, the pycnometer was filled with a liquid with known density, namely hexane, in which the sugar is assumed completely insoluble. The weight of the displaced hexane can then be determined, and subsequently the volume of sugar can also be calculated. Measurements were done in triplicate.

The bulk density of the sugars was analysed using a powder tester (Hosokawa Iron Works, Japan). During measurement, sugars were gently poured into the hopper of the machine which then automatically transferred into a cup below the hopper. A scraper was used to manually remove the sugar mound from the cup. This process was done in a very careful manner in order to create a flat surface with the same level as the edge of the cup. The bulk density of the powder was determined by measuring the ratio of mass to the volume occupied by the sugar.

3.3.2.8 Colour

The colour of the sugars was measured with a colorimeter (Minolta Model CM-2500D Spectrophotometer, Tokyo, Japan) which was calibrated using a white reference standard. The SCE-mode (Specular light excluded) values were recorded and the colour parameters were expressed in L*a*b* colour space system where L* represents lightness (luminance ranging from 0 (black) to 100 (white)), a* represents green to red, and b* represents blue to yellow. Measurements were done in triplicate.

3.3.2.9 Particle size distribution (PSD)

The PSD of the sugar (refractive index 1.54) was analysed using a Malvern Mastersizer S Long Bench (Malvern Instruments Ltd., Worcestershire) equipped with a 1000 F lens. This lens can measure sugar particle size ranging from 5-3500 µm. Additional devices consisting of a vibrating hopper (MS-64) to circulate the coarse sugar were installed with the air pressure was set at 2 bar. Approximately 100 g of sugar was used in each measurement. Measurements were done in triplicate. D(v,0.9), D(v,0.5), D(v,0.1), D(4,3) and D(3,2) and span, representing respectively a percentile of 90%, 50% and 10%, volume-weighed mean, Sauter diameter and distribution width, respectively, were derived from the result of the measurement.

3.3.2.10 Thermal behaviour

Thermal behaviour of the sugars was recorded with a Q1000 differential scanning calorimeter (DSC) equipped with a refrigerated cooling system (TA Instruments, New Castle, USA). The DSC was calibrated with indium, azobenzene and undecane. Approximately 2 mg of the sugar was hermetically sealed in an aluminium cup. Afterwards, the sample was equilibrated at 20^{o}C followed by a heating step to 200^{o}C at a rate of 5^{o}C/min to measure the melting profile.

For glass transition temperature measurement, the sample in a sealed pan was equilibrated at 20^{o}C followed by heating at a rate of 5^{o}C/min until the sample melted completely. Afterwards, the sample was quickly cooled to −40^{o}C at a rate of 5^{o}C/min and finally reheated at a rate of 5^{o}C /min to 210^{o}C. Measurements were done in triplicate. Melting profile and glass transition data integration was carried out with Universal Analysis 2000 software version 4.7A (TA Instruments) using linear baseline. Melting peaks were characterised by onset temperature (°C), maximum melting temperature (°C) and enthalpy (J/g). Glass transition was characterised by Onset (°C), Midpoint (°C), Offset (°C) and Δ Cp (J/(g·°C)).

3.3.2.11 Microstructural images

The surface morphology of sugars was visualised using a JSM-7100 F TTLS LV TFEG-SEM (Scanning Electron Microscopy) (Jeol Europe, Zaventem, Belgium) under high vacuum and at an accelerated voltage of 3 keV. Prior to electron beam targeting, the samples were vitrified in liquid nitrogen and transferred to a PP3000T device (Quorum Technologies, East Sussex, UK) at -140°C. Subsequently, the samples were allowed to sublime for 15 min at -70°C in order to remove frost, followed by sputtering a thin platinum film on the sample surface.

3.3.3 Data analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). The psychochemical properties of sugars were subjected to variance analysis (ANOVA) at 5% significance level. Testing for homogeneity of variances was performed with Levene Test. When the conditions for homogeneity of variances were fulfilled, Tukey test was used to determine differences among the samples. In case variances were not homogeneous, Games–Howell test was performed. Principal component analysis (PCA) was used to visualise the relationships between chemical and physical properties of the sugars.

3.4 RESULTS AND DISCUSSION

3.4.1 Chemical properties of palm sap sugars

Moisture, fat, protein, ash, reducing sugars and sucrose content are chemical properties of palm sap sugar expected to highly influence the characteristics of chocolate. Hence, in this study, the emphasis was put on the analyses of those chemical properties.

3.4.1.1 Moisture content

As discussed in Chapter 1 (Section 1.5.1), moisture content highly affects the quality attributes of chocolate, mainly rheological behaviour and textural properties (Afoakwa, 2010; Beckett, 2009). Apart from this, the excess moisture may also induce sugar bloom, thus reducing the appearance quality (Delbaere et al., 2016) and create sugar lumps affecting the fineness of chocolate (Beckett, 2008). The moisture content of chocolate ingredients should be maintained as low as possible because if approximately 0.3% moisture left in the chocolate, 1% cocoa butter should be added to create good quality of chocolate (Beckett, 2008).

Table 3.1 shows that coarse coconut sugar (CCS1, CCS2) and coarse palm sugar (CPS1, CPS2, CPS3) had significantly higher ($p<0.05$) moisture content, in the range of 1.0% to 2.4%, than that of coarse pure sucrose (CS), which contained only 0.3% moisture. It is also worth mentioning that CPS 2 exhibited significantly lower ($p<0.05$) moisture content than other palm sap Sugars, while CPS3 had the highest moisture content among all sugars.

To produce coarse palm sap sugar, the sap/nectar is boiled under agitation until crystalline sugar is formed. In this process, the duration, temperature and agitation method applied highly influence the degree of crystallinity and moisture left in the product. Afterwards, drying is required to evaporate moisture present in the final sugar. Different drying techniques used by either small-scale industries or farmers result in different levels of moisture (Phaichamnan et al., 2010a). Moreover, the applied grinding process of the dried sugars to reduce the particle size of the sugar may also create amorphous parts which are hygroscopic, contributing to a higher moisture absorption. Therefore, it is understandable why the moisture content of palm sap sugars was significantly higher ($p<0.05$) than that of pure crystalline sucrose and varied among samples (Table 3.1). During distribution, a hermetic packaging is highly required to avoid further moisture absorption from the environment. High moisture level tends to make sugar particles to stick together, thus reducing the quality of coarse palm sugar.

Table 3.1. Chemical composition of sampled palm sap sugars (dry matter basis)

Sugar	Moisture(%)	Protein (%)	Ash (%)	Sucrose (%)	Reducing sugars	
					Fructose (%)	Glucose (%)
CCS1	1.8 ± 0.1^{cd}	1.2 ± 0.0^{c}	2.0 ± 0.0^{d}	85.0 ± 2.1^{ab}	2.4 ± 0.1^{b}	1.2 ± 0.1^{b}
CCS2	1.9 ± 0.1^{d}	0.8 ± 0.0^{b}	1.5 ± 0.0^{c}	81.9 ± 4.5^{a}	2.7 ± 0.1^{c}	2.0 ± 0.0^{c}
CPS1	1.7 ± 0.1^{c}	1.6 ± 0.0^{e}	2.0 ± 0.0^{e}	84.1 ± 1.5^{a}	1.4 ± 0.0^{a}	0.7 ± 0.0^{a}
CPS2	1.0 ± 0.1^{b}	0.5 ± 0.0^{a}	0.3 ± 0.0^{b}	95.2 ± 6.0^{b}	1.3 ± 0.1^{a}	1.3 ± 0.1^{b}
CPS3	2.4 ± 0.1^{e}	1.3 ± 0.0^{d}	2.1 ± 0.0^{f}	78.7 ± 3.5^{a}	3.3 ± 0.2^{d}	2.0 ± 0.1^{c}
CS	0.3 ± 0.0^{a}	n.d.	0.0 ± 0.0^{a}	100	n.d.	n.d.

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples
n.d. : not detected

3.4.1.2 Fat content

As previously discussed in Chapter 1 (Section 1.5.2), fat is responsible for dispersing the other ingredients. The presence of more fat in chocolate, due to the addition of ingredients containing fat, influences the particle volume fraction, which further affects the chocolate characteristics, particularly the ones influenced by particle-particle interaction (Afoakwa, 2010; Beckett, 2009). Moreover, as described in Chapter 1 (Section 1.3.2), the presence of another type of fat with different triacylglycerol (TAG) composition also affects the crystallisation behaviour of chocolate. Thus, the fat content analysis was carried out to verify that fat was not present in palm sap sugars. As expected, fat was not detected in the palm sap sugars.

3.4.1.3 Protein content

Protein/amino acids are present not only in the sap/nectar, but also in palm sap sugar (Ho et al., 2008b; Purnomo, 2007). Thus, in order to have a better understanding on the potency of protein found in the palm sap sugar in inducing additional Maillard reaction, protein content analysis was carried out. In the chocolate production, chocolate is conched at 50-80^{o}C for hours, depending on the type of chocolate and sugar used (Beckett, 2009). This process may induce additional Maillard reaction which in turn may create distinctive aroma and colour of chocolate (Chapter 2; section 2.5.8).

As can be seen in Table 3.1, the results showed that protein was present in coarse coconut sugar (CCS1, CCS2) and coarse palm sugar (CPS1, CPS2, CPS3), in contrast to sucrose (CS). The protein level varied among samples in the range of 0.5% to 1.6%. Different protein contents among palm sap sugars may be due to different composition of the sap/nectar as well as the time and temperature employed during palm sap sugar production. These factors determine the degree of Maillard reaction occurred.

3.4.1.4 Ash content

Palm sap sugar contains various minerals, such as phosphorus, potassium, calcium, magnesium, iron *etc* (Arcieri, 2014; Philippine Coconut Authority, 2016). Thus, in order to be able to roughly quantify them, ash content analysis was conducted. As stated by Marshall (2010), ash content represents the total mineral present in foods. It can be seen in Table 3.1 that palm sap sugars, in contrast with sucrose, contained relatively high ash ranging from 0.3-2.1% indicating the presence of high minerals. In more detail, CCS1, CPS1 and CPS3 exhibited higher ash content than CCS 2 and CPS 2, while there was no ash present in sucrose, indicating its high purity. The presence of impurities affects the degree of palm sap sugar crystallinity (Section 3.4.2.5.1), which in turn affects the quality attributes of chocolate, more particularly fineness, rheological and textural properties.

3.4.1.5 Sucrose and reducing sugars content

Sugar composition analysis was carried out to measure the amount of sucrose and reducing sugars (fructose, glucose, lactose and maltose) in palm sap sugar. Similar to the protein content, the amount of reducing sugars, which is a Maillard reaction precursor, in palm sap sugar was highly influenced by the degree of Maillard reaction occurring during sugar production. The amount of fructose and glucose in sap/nectar decrease as the degree of Maillard reaction increases (Apriyantono et al., 2002; Ho et al., 2008b; Naknean et al., 2009b). However, the conversion of sucrose to fructose and glucose during sugar production (Phaichamnan et al., 2010a) should also not be neglected. Table 3.1 shows that fructose and glucose were only present in palm sap sugars (CCS1, CCS2, CPS1, CPS2 and CPS3) and in varying amounts (fructose: 1.3-3.3% and glucose: 0.7-2.0%). As expected, lactose and maltose, which is commonly present in milk and starch, respectively (Beckett, 2009; Lees, 2012) were not found in the palm sap sugars. The presence of glucose and fructose may induce additional Maillard reaction during chocolate production which may create chocolate with distinctive colour and aroma characteristics. Moreover, these sugars may have a positive influence in improving the heat stability of chocolate due to the formation of a network of matted sugar particles (Beckett, 2009). However, the use of glucose and fructose which are very hygroscopic may adversely affect the flowability and the fineness of chocolate (Beckett, 2008).

3.4.2 Physical properties of palm sap sugars

3.4.2.1 Solubility

From the chocolate processing point of view, the solubility of sweetener is very important. High solubility of sweetener can result in gritty agglomerates at high conching temperatures (Beckett, 2009). This may affect the quality attributes of chocolate, particularly fineness, rheological and textural properties.

As can be seen in Table 3.2, coarse coconut sugar (CCS1, CCS2) and coarse palm sugar (CPS1, CPS2 and CPS3) possessed significantly higher ($p<0.05$) solubility (67-70%) than sucrose, CS, (66%). This phenomenon can be attributed to the presence of minerals, reducing sugars, proteins, and moisture in palm sap sugar (Asadi, 2006; Lees, 2012; Qian et al., 2010; Sun et al., 2012). Theoretically, pure sucrose has a solubility of 66.5% at room temperature (20^{o}C) (Hartel et al., 2011). Changes in the molecular interaction between the solutes and the water due to the presence of impurities might be responsible for the increase in solubility (Asadi, 2006; Hartel and Shastry, 1991; Lees, 2012). Apart from the impurities, some amount of amorphous sugar which can be generated during palm sap sugar production also increase the solubility to some extent. The fact that amorphous sugar is less physically stable than the crystalline sugar can explain this phenomenon (Qian et al., 2010; Sun et al., 2012).

3.4.2.2 Particle density and bulk density

Particle density of ingredient is very crucial in chocolate manufacture. In a given chocolate mass, the particle volume fraction (Chapter 1; section 1.5.2) is determined by particle density of the solid particles. The lower the particle density, the higher the particle volume fraction will be. In turn, it determines the quality attributes of chocolate, mainly the rheological and textural properties. It can be seen in Table 3.2 that particle density of sucrose (CS) was significantly higher ($p<0.05$) than that of palm sap sugars (CCS1, CCS2, CPS1, CPS2 and CPS3). Theoretically, refining/milling in the chocolate production will not change the particle density of sugars, this means that in a given chocolate mass, palm sap sugar-sweetened chocolate has higher particle volume fraction than sucrose-sweetened chocolate.

It can also be seen in Table 3.2 that the trend in particle density and bulk density of the sugars were similar. The higher the particle density, the higher the bulk density was observed. Eventhough the bulk density of the sugars is changed after refining/milling step in the chocolate production, it can be seen that sucrose (CS), which had quite similar particle size distribution to palm sap sugars (CPS1, CPS2) (Table 3.3), had higher bulk density than CPS1 and CPS2. These results emphasised that palm sap sugars are bulkier than sucrose. The bulkiness of chocolate ingredients highly influences the characteristics of chocolate, as previously discussed in Chapter 2.

Table 3.2 Physical properties of palm sap sugar which may influence the characteristics of chocolate

Sugar	Solubility (%)	Bulk Density (g/mL)	Particle Density (g/cm^3)	Colour		
				L*	a*	b*
CCS1	69.8 ± 0.3[b]	0.6 ± 0.0[a]	1.52 ± 0.01[a]	48.2 ± 0.7[c]	17.6 ± 0.8[c]	67.2 ± 2.5[c]
CCS2	69.8 ± 0.3[b]	0.6 ± 0.0[d]	1.53 ± 0.01[a]	47.7 ± 0.7[c]	16.5 ± 0.2[c]	62.1 ± 1.2[b]
CPS1	69.7 ± 0.5[b]	0.7 ± 0.0[c]	1.56 ± 0.01[bc]	38.2 ± 0.2[a]	19.7 ± 0.9[b]	61.8 ± 0.5[b]
CPS2	67.0 ± 0.3[a]	0.7 ± 0.0[c]	1.56 ± 0.01[bc]	36.8 ± 1.2[a]	20.7 ± 0.2[b]	59.7 ± 1.4[b]
CPS3	69.9 ± 0.1[b]	0.6 ± 0.0[b]	1.54 ± 0.01[ab]	41.8 ± 0.3[b]	17.4 ± 0.5[c]	61.9 ± 1.7[b]
CS	66.3 ± 0.1[a]	0.9 ± 0.0[e]	1.57 ± 0.01[c]	95.2 ± 0.1[d]	0.0 ± 0.0[a]	1.1 ± 0.3[a]

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

3.4.2.3 Colour

During palm sap sugar production, Maillard reaction and caramelisation take place indicated by the formation of brown coloured sugar, with the colour becoming darker as the heating time increases (Apriyantono et al., 2002; Ho et al., 2008b; Naknean and Meenune, 2011a; Naknean et al., 2009a). Colour measurement showed that coarse palm sap sugars exhibited much more intensive brown colour compared to sucrose which was indicated by a lower L* and higher a* and b* values (Table 3.2). L* indicates the lightness of the sugar, while positive value of a* and b* indicate the redness and yellowness of the sugar. The variation in the colour parameter values among palm sap sugars was associated with the degree of Maillard reaction of palm sap sugar.

The colour differences among sugars can be visually seen in Figure 3.1. Cursory inspection indicated visually that CPS 1 and CPS2 had darker colour than CCS1, CCS2 and CPS3, while, in general, palm sap sugars were much darker than sucrose.

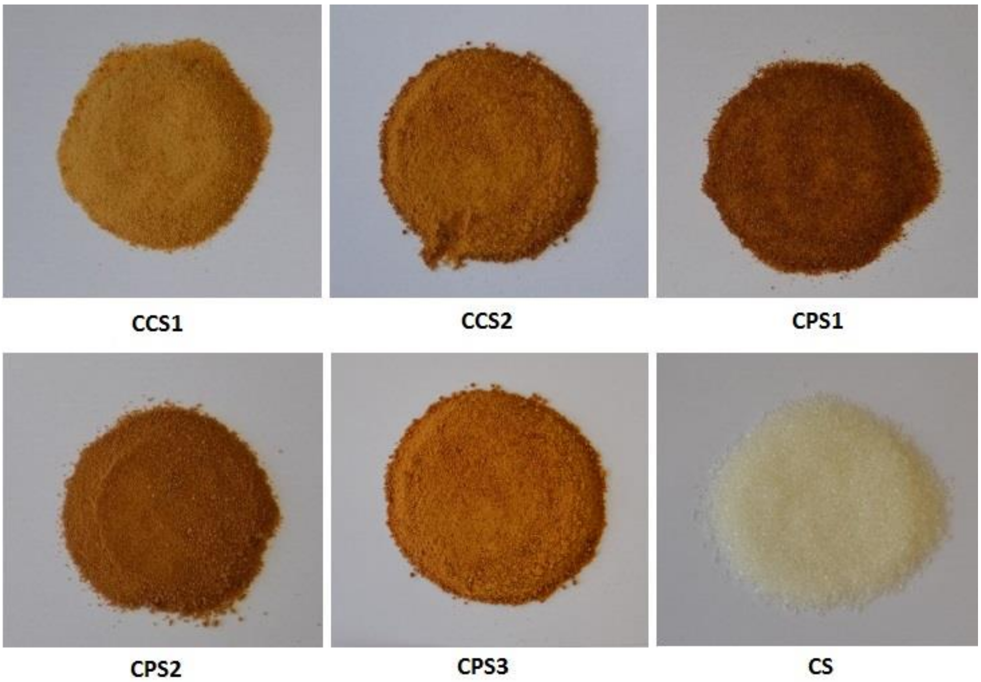


Figure 3.1. The pictures of coarse palm sap sugars and sucrose

3.4.2.4 Particle size distribution (PSD)

The PSD of sugar is a very important aspect that should be investigated. The PSD is used to determine the duration of milling or the rollers gap applied during refining process in the chocolate production. Moreover, the PSD values can also be used to describe the homogeneity of the sugar particles. It can be seen in Table 3.3 that the PSD values of palm sap sugars (CCS1, CCS2, CPS1, CPS2 and CPS3) varied not only in terms of the largest size of the particle D(v,0.9), but also of D(v,0.5), D(v,0.1), D(4.3), and D(3.2). The variation was attributed to the different method of sugar production used by farmers. Aside from this, particle agglomeration was highly likely to occur in the presence of relatively high moisture as well as reducing sugars (glucose and fructose) in the palm sap sugar which may present in the form of amorphous phase, thus affecting the measured PSD. Moreover, as expected, coarse sucrose had more uniform particle size than palm sap sugars which can be seen from their span value (Table 3.3). A uniform particle size of sugar is important to easily determine the milling duration during chocolate production. The span value of CCS1, CCS2, CPS1, CPS2 and CPS3 (1.7-2.5) were wider than that of CS (1.4) indicating the presence of groups of big and small particles. The fact that palm sap sugar is still traditionally produced by farmers without a proper sieving process was the reason for this observation. In general, eventhough the distribution profile was different, the D(v,0.9) value of sucrose was still in the range of those of palm sap sugars (900 to 1450 µm).

Table 3.3 Particle size distribution of palm sap sugar as compared to that of sucrose

Sugar	Distribution Percentiles (µm)			Derived Diameter (µm)		Span (-)
	D(v, 0.9)	D(v, 0.5)	D(v, 0.1)	D(4,3)	D(3,2)	
CCS1	1249 ± 88^{c}	556 ± 56^{b}	167 ± 15^{b}	640 ± 46^{b}	267 ± 26^{a}	2.0 ± 0.2^{c}
CCS2	1182 ± 70^{b}	423 ± 45^{a}	122 ± 4^{a}	549 ± 40^{a}	206 ± 7^{a}	2.5 ± 0.1^{d}
CPS1	925 ± 75^{a}	452 ± 33^{a}	141 ± 15^{ab}	497 ± 38^{a}	216 ± 23^{a}	1.7 ± 0.1^{b}
CPS2	900 ± 101^{a}	443 ± 18^{a}	165 ± 1^{b}	497 ± 42^{a}	235 ± 2^{a}	1.7 ± 0.2^{b}
CPS3	1171 ± 16^{b}	568 ± 12^{b}	200 ± 3^{c}	633 ± 10^{b}	367 ± 7^{b}	1.7 ± 0.0^{b}
CS	926 ± 14^{a}	504 ± 10^{a}	222 ± 8^{d}	542 ± 10^{a}	371 ± 26^{b}	1.4 ± 0.0^{a}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

3.4.2.5 Thermal properties

Sugar plays a very important role in determining the quality attributes of chocolate and foods in general. Therefore, understanding the state of the sugar is very crucial. In the chocolate matrix, sugar may be present in crystalline state or amorphous (glassy) state or their combination. During processing, sugar in the confectionery formulations can undergo one or more phase transition, depending on the nature of the product (Hadjikinova and Marudova, 2016; Hartel et al., 2011). Hence, in order to elucidate the influence of palm sugar on the chocolate characteristics, melting and glass transition temperature were studied.

3.4.2.5.1 Melting profile

Melting is an endothermic transition where the crystalline state transforms to the liquid state upon heating (Hadjikinova and Marudova, 2016). The presence of impurities, such as water and other chemical compounds, lowers the melting temperature of a substance (Beckett et al., 2006 ; Kedward et al., 1998a; Roos, 1993b; Roos and Karel, 1991). Figure 3.2

shows the melting curve of the sugars. It can be seen that coarse coconut sugars (CCS1, CCS2) and palm sugars (CPS1, CPS2 and CPS3) in general had a blunt, broad, and asymmetric peak with large width values (13.2-20.7^{o}C), while coarse sucrose had a sharp and symmetric peak with very small width value (2.9^{o}C). Furthermore, it can be seen in Table 3.4 that palm sap sugars (CCS1, CCS2, CPS1, CPS2, and CPS3) exhibited significantly lower ($p<0.05$) onset (138.0-162.3^{o}C) and melting peak temperatures (160.2-183.0^{o}C) than sucrose (CS) which exhibited onset and melting peak temperature of 187.9^{o}C and 189.5^{o}C, respectively. A higher content of impurities and moisture, which act as plasticiser, in palm sap sugars were responsible for this phenomenon. Coarse sucrose (CS) which had a very high purity exhibited comparable melting temperature to sucrose studied in the other works with the value fell within the range of 185 to 190^{o} C (Hurtta et al., 2004; Roos, 1993b). Moreover, it can also be seen that the enthalpy value of CS (154.4 J/g) was higher than that of CCS1, CCS2, CPS1, CPS2, and CPS3 (87.7-119.9 J/g), indicating that a higher energy was required to melt the sugar crystals. This further showed that sucrose had a higher amount of crystalline part than palm sap sugars. In the palm sap sugars, some amorphous parts present were most likely formed during their production. Many food materials exist in a completely or partially amorphous state due to food processing (Kim et al., 2001; Liu et al., 2006b; Meste et al., 2002).

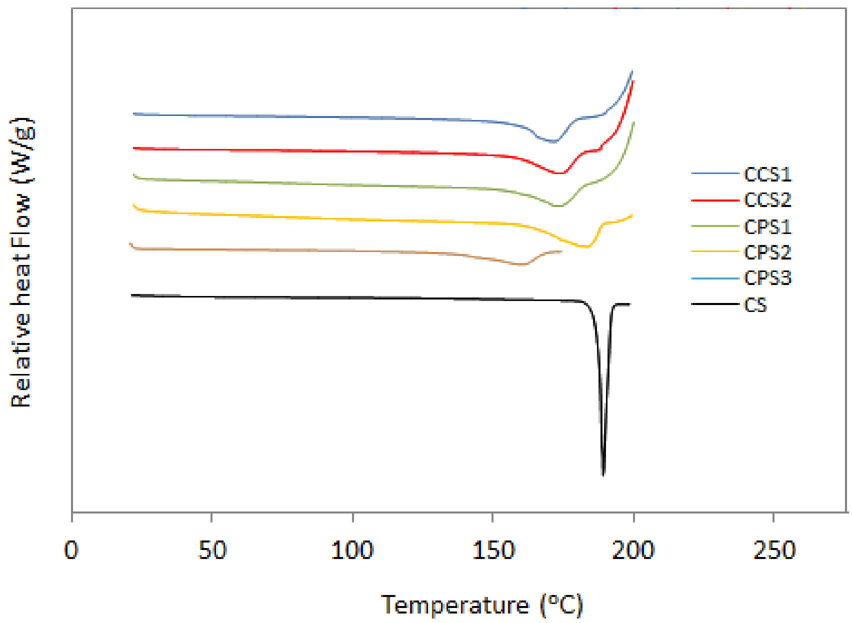


Figure 3.2. Melting point of palm sugar as compared to that of sucrose

Variation of the melting profile within palm sap sugars was attributed to the different level of impurities present in the sugars. For example, CPS3, which had higher content of moisture, reducing sugars, protein and ash than other palm sap sugars, exhibited the lowest melting temperature and enthalpy values (Table 3.4). Regarding the onset temperature or the temperature when the sugar starts to melt, its variation was not only due to the presence of different amount of impurities, but also due to the different thermal contact between the sugar and the DSC pan. The latter factor may result in different temperature recorded during thermal profiling.

Table 3.4. Melting profile of palm sugar as compared to that of sucrose

Sugar	Onset (°C)	Melting peak (°C)	Width (°C)	Enthalpy (J/g)
CCS1	160.6 ± 3.0^{bc}	171.9 ± 1.3^{b}	13.2 ± 0.9^{b}	107.7 ± 12.0^{b}
CCS2	156.8 ± 1.8^{b}	174.3 ± 0.6^{c}	14.8 ± 0.6^{bc}	119.9 ± 6.9^{b}
CPS1	158.2 ± 0.3^{bc}	173.8 ± 0.9^{bc}	16.2 ± 0.6^{c}	118.3 ± 0.7^{b}
CPS2	162.3 ± 0.2^{c}	183.0 ± 0.2^{d}	16.4 ± 0.3^{c}	118.8 ± 0.5^{b}
CPS3	138.0 ± 2.3^{a}	160.2 ± 0.8^{a}	20.7 ± 0.8^{d}	87.7 ± 6.2^{a}
CS	187.9 ± 0.2^{d}	189.5 ± 0.4^{e}	2.9 ± 0.1^{a}	154.4 ± 1.9^{c}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

3.4.2.5.2 Glass transition

Most of the pure solid sugars are present in the crystalline state, while in impure sugar some amount of amorphous state may also be present (Kasapis, 2005). Amorphous state is widely known to undergo structural changes due to the presence of moisture and/or heat, resulting in undesirable events, such as stickiness, caking, and collapse. The amorphous state can exist either as a viscous fluid-like rubbery state or as a highly viscous glassy state with low molecular mobility (Hadjikinova and Marudova, 2016; Liu et al., 2006b). The glass transition occurs when the glassy state converts to the rubbery state (or vice versa) (Ergun et al., 2010; Hadjikinova and Marudova, 2016; Lee et al., 2011; Meste et al., 2002). Amorphous sugar is highly hygroscopic and will absorb moisture from the environment, resulting in plasticisation that lowers the glass transition temperature (Abbas et al., 2010). In the chocolate matrix, the presence of amorphous state may create problems during chocolate processing and storage, especially with regard to the rheological and textural changes (Hanselmann, 2013). However, the presence of amorphous state may increase the intensity of cocoa flavour (Beckett, 2009).

Due to the presence of glucose and fructose as well as moisture, the glass transition temperature of palm sap sugars were expected to be lower than that of sucrose, but higher than that of glucose and fructose. The glass transition temperature of a mixture is usually located between the glass transition temperatures of its components (Hadjikinova and Marudova, 2016; Liu et al., 2006b; Seo et al., 2006a). In this work, the measurement of glass transition temperature was initially conducted by directly heating the sugar samples from 20°C to 200°C at a rate of 5°C/min. However, the glass transition could not be detected which may be attributed to relatively small amount of amorphous part present in palm sap sugars. Aside from this, the low sensitivity of the instrument may also contribute to this phenomenon.

To roughly estimate the glass transition temperature of the palm sap sugars, a different method following the description of Liu et al. (2006a) and Saavedra-Leos et al. (2012) with slight modifications was chosen. This method involves the melting of crystalline state followed by fast cooling to create glass state. Afterwards, the glass transition temperature was measured through re-heating the created sugar glass (Liu et al., 2006b). The drawback of this method is that the thermal history of the sugars is changed. Moreover, caramelisation may occur during the first heating, which results in the degradation of some sugar molecules and polymerisation (Jiang et al., 2008), thus influencing the measured glass transition to some extent.

Regardless of molecule degradation and polymerisation that may occur due to "heating-cooling-reheating" method, the glass transition temperature is influenced by heating rate, holding time, and final first heating temperature (Jiang et al., 2008; Vanhal and Blond, 1999). Thus, the fixed glass transition temperature of a material is often difficult to determine. For instance, the glass transition temperature of sucrose reported by different authors varies between 62 °C and 75°C (Hadjikinova and Marudova, 2016; Jiang et al., 2008; Vanhal and Blond, 1999).

The glass transition takes place at a range of temperatures. Thus, in this study, the glass transition temperature is reported as the onset, midpoint and endpoint/offset temperature. Regardless of the thermal history of the sugars, the glass transition temperature of palm sap sugars (CCS1, CCS2, CPS1, CPS2 and CPS3) were significantly lower than that of sucrose (CS) as expected. It can be seen in Table 3.5 that CS had a glass transition temperature in the range of 62.6 - 69.4 °C, which was comparable to that of sucrose reported by Roos (1993a) and Hartel et al. (2011) (62 - 72 °C). Variation of the glass transition temperature within palm sap sugars was attributed to the variation on the amount of impurities and different level of molecule degradation and polymerisation that occur during the first heating. Regarding the specific heat (ΔCp), it can be seen that the values among samples were not significantly different (p>0.05), with the specific heat of CPS1 being slightly higher than the other samples.

Table 3.5. Glass transition parameters of palm sugar as compared to that of sucrose

Sugar	Glass Transition Temperature (Tg)			
	Onset (°C)	Midpoint (°C)	Offset (°C)	Δ Cp (J/(g·°C))
CCS1	54.0 ± 0.7^{c}	57.7 ± 1.4^{d}	61.0 ± 0.0^{c}	0.4 ± 0.0^{ab}
CCS2	54.2 ± 1.9^{c}	58.5 ± 1.8^{d}	63.1 ± 1.7^{c}	0.3 ± 0.0^{a}
CPS1	35.9 ± 1.3^{a}	39.9 ± 1.2^{a}	44.3 ± 0.6^{a}	0.6 ± 0.0^{b}
CPS2	46.9 ± 0.4^{b}	50.6 ± 0.7^{c}	54.2 ± 0.9^{b}	0.4 ± 0.0^{ab}
CPS3	41.2 ± 4.4^{b}	44.9 ± 3.4^{b}	48.3 ± 3.2^{a}	0.2 ± 0.0^{a}
CS	62.6 ± 0.9^{d}	67.0 ± 0.3^{e}	69.4 ± 1.0^{d}	0.3 ± 0.0^{a}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences (p < 0.05) among samples

3.4.2.6 Microstructural images

Microstructural visualisation of sugars was carried out to study the surface morphology of the crystal sugar. The results revealed (Figure 3.3) that the surfaces of palm sap sugars, in contrast to sucrose, were covered with layers which induce the sugar crystals to stick to each other, creating agglomerates. The presence of these layers may be due to the relatively high moisture content (Beckett, 2008), as well as due to the presence of hygroscopic materials such as fructose, glucose and amorphous sugar (Saputro et al., 2017).

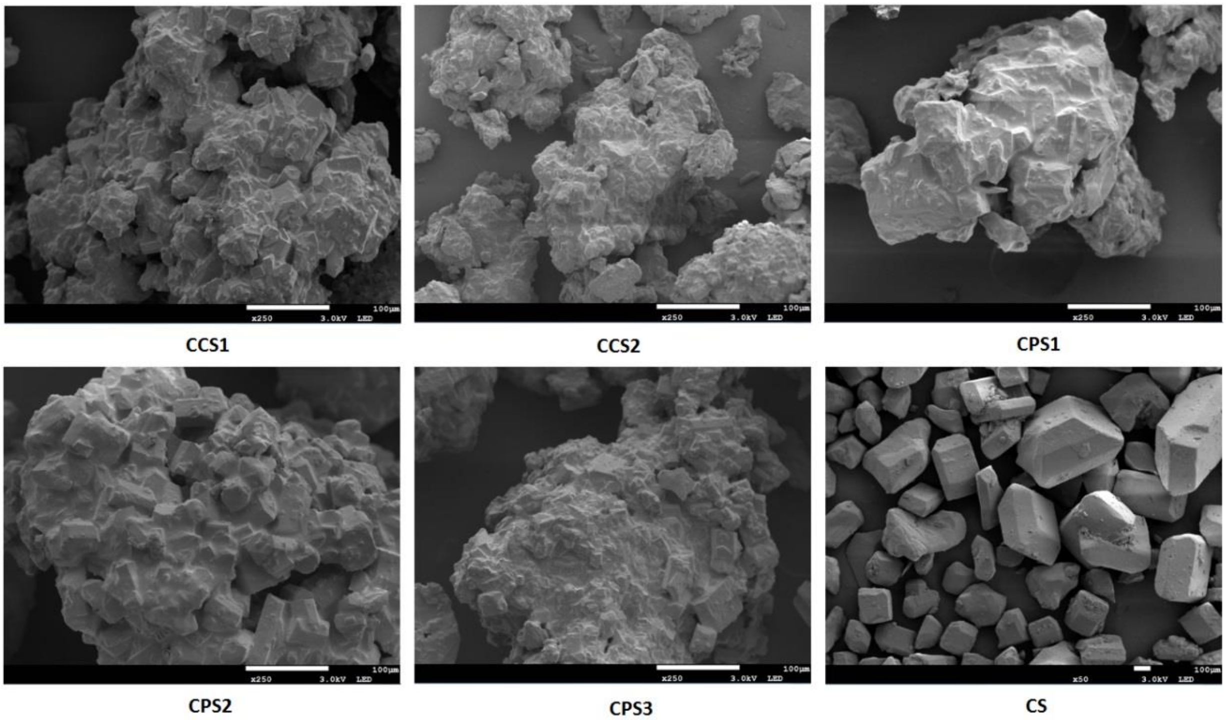
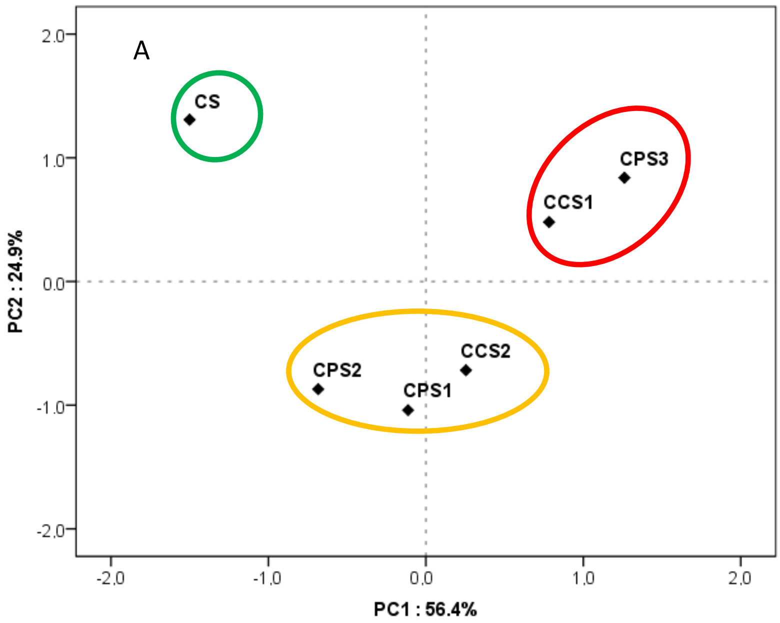


Figure 3.3 Surface morphology of sugars.
Scale bar represents 100 µm (CCS1, CCS2, CPS1, CPS2, CPS3) and 10 µm (CS).

3.4.3 Interconnection between chemical and physical properties of palm sap sugars

The interconnection between chemical and physical properties of palm sap sugar was investigated using principal component analysis (PCA). It can be seen in Figure 3.4 that PCA explained more than 81% variance in the first two factors, namely PC1: 56.4% and PC2: 24.9%. From the Figure 3.4 (A), it can be observed that there were 3 clusters of sugar. The first cluster (CCS1, CPS3), characterised by high positive values in PC1, exhibited high moisture, fructose, protein and ash content. Moreover, this cluster also exhibited high D(v,0.9), solubility and width values which can be attributed to the presence of aforementioned chemical compounds, as discussed in Chapter 2 (Section 3.4.2). The second cluster (CPS1, CPS2, CCS2), characterised by relatively high negative values in PC2, exhibited high moisture, glucose, protein and ash content. In turn, these chemical compounds resulted in palm sap sugar with high solubility, span, width, a and b values. The third cluster (CS), characterised by high negative value in PC1 and high positive value in PC2, exhibited high sucrose content and L value. Due to its high purity, this cluster also exhibited high enthalpy value, melting and glass transition temperature. Moreover, this cluster also exhibited high particle and bulk density. Figure 3.4 (B) shows that, in general, the higher the chemical impurities (moisture, fructose, glucose, protein, ash), the lower the enthalpy value, melting temperature, glass transition temperature, bulk and particle density were observed. Moreover, the higher chemical impurities, the higher D(v,0.9), solubility, span, width, a* and b* values were observed.



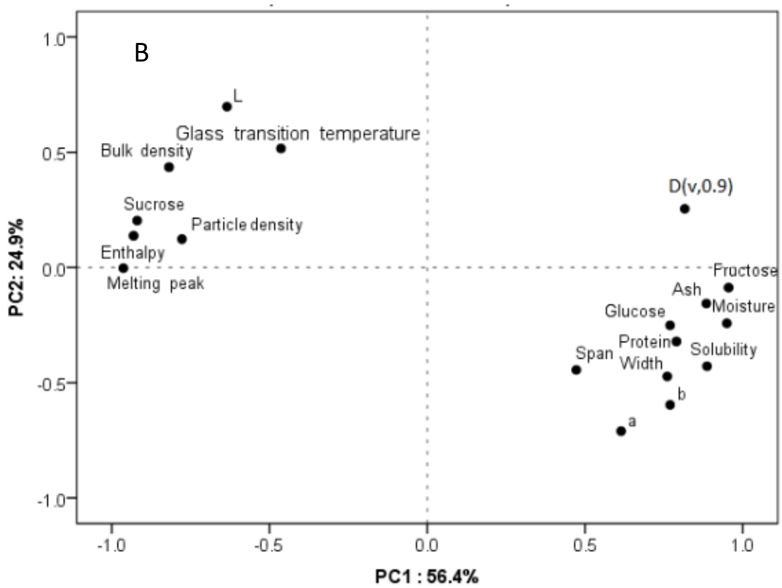


Figure 3.4 PCA score plot (A) of physicochemical properties of sugars. PCA loading plot (B) of palm sap sugars and sucrose.

3.5 CONCLUSIONS

Several palm sap sugars used in this study exhibited various physicochemical properties. This result can be attributed to the differences on the raw materials and processing methods used by the sugar producer. The degree of non-enzymatic reactions that occur during sugar production, namely Maillard reaction and caramelisation, was responsible for the different level of protein and reducing sugars present in the palm sap sugars. These compounds together with the presence of moisture and minerals determined the level of palm sap sugars' purity. Furthermore, in combination with thermal and mechanical processes applied during sugar production, they defined the solubility, thermal behaviour, particle density, and particle size distribution of coarse palm sugar.

The use of palm sap sugar as chocolate sweetener will definitely affect the quality characteristics of chocolate. Moisture and reducing sugars together with particle density and particle size distribution are expected to affect the rheological and textural characteristics of chocolate. Moreover, the presence of relatively high moisture may induce agglomeration of sugar which in turn may affect the fineness of chocolate. Apart from this, the presence of relatively high moisture, reducing sugars and proteins in the palm sap sugar may induce additional Maillard reaction during chocolate production. If the additional Maillard reaction occurs, it can generate the distinctive aroma profile and colour of chocolate. In addition, the presence of amorphous phase in palm sugar may result in chocolate with more intense flavour. It seems that the use of palm sugar will not influence the melting behaviour of chocolate.

Nevertheless, the extent of the influence of palm sugar on the chocolate characteristics should be further investigated. Subsequently, adjustment in the formulation and chocolate processing method can be applied where needed to obtain palm sap sugar sweetened chocolate with the desired quality characteristics.

CHAPTER 4

IMPACT OF PROCESSING VARIABLES ON THE RHEOLOGICAL BEHAVIOUR AND MICROSTRUCTURAL PROPERTIES OF DARK CHOCOLATE PRODUCED BY COMBINATION OF A BALL MILL AND A LIQUEFIER DEVICE

This chapter is prepared for publication :
Saputro, A.D., Van de Walle, D., Caiquo, B.A., Kluczykoff, M., Dewettinck, K. Impact of processing variables on the rheological behaviour and microstructural properties of dark chocolate produced by combination of a ball mill and a liquefier device.

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4.1 ABSTRACT

An alternative chocolate processing method, which can be applied by either small-scale chocolate industries or farmers in cocoa producing countries, is highly needed. This work investigated the impact of processing variables and lecithin concentration on the rheological behaviour and microstructural characteristics of dark chocolate produced by means of a ball mill coupled with a liquefier device. Three experimental variables, namely ball size, milling time and lecithin concentration were studied. To investigate the degree of cocoa butter released from the cocoa particles during the chocolate production, two different cocoa sources, which had different level of cocoa butter, namely cocoa mass and cocoa powder, were used as ingredient. As references, chocolates were also conventionally produced using a combination of a planetary mixer, roll-refiner and conche. The results showed that regardless of the lecithin concentration, ball size, milling time and their interaction were the most decisive experimental variables in the milling process. Furthermore, lecithin concentration, ball size and milling time as well as their interactions highly influenced the rheological behaviour of the chocolates. Moisture and free fat content of the chocolates were comparable regardless of the processing method and the type of cocoa used. Regardless of the cocoa source, Casson yield values of the chocolates produced by alternative processing were higher than those of the chocolates produced by conventional processing, while Casson viscosities were relatively similar. From a rheological and microstructural perspective, irrespective of the ball size, the alternative processing with 30 min milling time and 0.4% lecithin, seems to be the most suitable method for small-scale chocolate industries.

4.2 INTRODUCTION

Chocolate is one of the most popular confectionery products in the world which is commonly produced using middle-big scale machines requiring high investment of capital (Afoakwa, 2010; Beckett, 2009). This is a disadvantage for developing countries such as Ghana, Indonesia, Ivory Coast, *etc* where cocoa as the main raw material for chocolate production is highly produced (International Cocoa Organisation (ICCO), 2014). Therefore, a small-scale chocolate production system which can economically be applied by farmers and small scale chocolate industries in cocoa producing countries is highly required.

Conventionally, molten chocolate is produced using a sequence of mixing, roll-refining and conching (Afoakwa, 2010; Beckett, 2009). The latter process is a very time and energy consuming stage, but is crucial since moisture reduction and fine-tuning of a desirable flavour profile occur during this processing step. Aside from this, the textural and rheological characteristics of chocolate are also developed in this stage (Afoakwa, 2010). A promising small-scale chocolate processing machine is the ball mill in which milling and conching-like process are carried out at the same time (Bolenz et al., 2014a), thus a compact production system for small chocolate industry can be created (Bolenz and Manske, 2013). In contrast to the conventional processing in which the chocolate production is sometimes carried out in an open container with high risk of contamination, the alternative processing using ball mill is conducted in a closed chamber (Bolenz et al., 2014a; Bolenz et al., 2014b). However, reducing moisture and undesired volatiles is more difficult by the latter method (Bolenz et al., 2014a; Bolenz and Manske, 2013; Bolenz et al., 2014b). Apart from that, the chocolate produced using a ball mill which should be able to flow during the entire milling process requires a higher fat content, not conducive to the aforementioned moisture reduction (Bolenz and Manske, 2013; Bolenz et al., 2014b).

Few researches related to the utilisation of a ball mill in chocolate production have been conducted. Alamprese et al. (2007) studied the optimisation of refining process in terms of time and energy consumption for dark chocolate production. Bolenz et al. (2013, 2014a, 2014b, 2014c) studied the impact of fat content, PSD and processing variables on milk chocolate properties. Pajin et al. (2013) investigated the crystallization and rheological properties of soya milk chocolate. Lucisano et al. (2006) studied the influences of formulation and processing variables on the physical characteristics of milk chocolate. Moisture is the main factor influencing the flow behaviour of chocolate. To cope with the difficulties in evaporating moisture, pre-dried materials were mostly used, especially with regard to high-moisture material such as milk powder (Bolenz and Manske, 2013; Bolenz et al., 2014c; Lucisano et al., 2006). Another potential method is the utilisation of a liquefier and/or a moisture removing device (Beckett, 2009; Lucisano et al., 2006). This device can be used to create a more homogeneous chocolate suspension after milling so that good flowability of molten chocolate can be achieved. Nevertheless, only a few studies have been reported on the use of a ball mill which is coupled with a liquefier device.

This research was aimed to investigate the impact of processing variables on the rheological behaviour and microstructural characteristics of dark chocolate produced using a ball mill coupled with a liquefier device as an alternative processing. With regard to this purpose, two strategies were set up. Firstly, to optimise the flowability of molten chocolate, extra lecithin was added in the stage prior to the liquefaction step. Secondly, during mixing/conching-like process cocoa butter was gradually added to maximise the reduction of undesirable flavour and moisture.

In the conventional chocolate processing, a conching machine which has wedge-shaped blades attached to the rotor smear the chocolate ingredient along the wall. After a certain time, the rotor is reversed in order to create a higher shearing action (Beckett, 2009) to allow some amount of cocoa butter to be released from the cocoa particles. The amount of the cocoa butter released depends on the shear, time and temperature (van der Vaart et al., 2013). In the alternative processing, there is no specific conching step. However, the milling process of the chocolate ingredient in the ball mill chamber can be considered as conching-like process (Bolenz et al., 2014a). Aside from this, mixing and liquefaction process using a liquefier device are also involved. Therefore, in order to get better insights into the release of cocoa butter during chocolate production, two different cocoa sources that have different fat level, namely cocoa powder and cocoa mass, were used as cocoa ingredient.

The performance of this alternative chocolate production system was investigated through moisture level analysis, free cocoa butter content, fineness (particle size distribution), rheological behaviour and microstructural characteristics of molten chocolate. The Moisture level, free cocoa butter content and fineness as the basic parameters which highly influence the rheological behaviour, can then be used to predict other quality attributes of chocolate such as colour (Afoakwa et al., 2008e; Saputro et al., 2016a), hardness (Afoakwa et al., 2008f; Saputro et al., 2016a; Stortz and Marangoni, 2011), aroma profile (Afoakwa et al., 2009a; Saputro et al., 2016a), and also thermal behaviour of the sugar phase (Saputro et al., 2016a; Saputro et al., 2016b) (Chapter 6; section 6.4).

Rheological behaviour is a very important quality attribute of chocolate. The consumer acceptance relies on the mouthfeel, which does not only depend on the particle size but also on the flow properties of molten chocolate (Bolenz et al., 2014a; Do et al., 2007). Furthermore, flow behaviour of molten chocolate is relevant for the handling of chocolate during its application (Afoakwa, 2010; Beckett, 2009). Microstructural characteristics of chocolate is also a crucial factor in determining not only the rheological behaviour but also other macroscopic properties of chocolate. In order to be able to objectively evaluate the alternative processing performance, chocolates produced by conventional processing were used as references.

4.3 MATERIALS AND METHODS

4.3.1 Raw materials

The raw materials used for the chocolate production were obtained in Belgium. Cocoa mass (CM1 IC B), cocoa butter (pure prime pressed cocoa butter CB 1) and soy lecithin (SLS-578-12) were purchased from Belcolade, Erembodegem. Cocoa powder (alkalised RMS 1801) was kindly supplied by Barry Callebaut, Wieze. Coarse sucrose (Kristalsuiker EU2) was obtained from Tiense Suikerraffinaderij, Tienen.

4.3.2 Sample Preparation

Dark chocolates with a total fat of 36% were produced using a combination of a ball mill and a Stephan mixer (Section 4.3.2.1) which had a production capacity of 2.5 kg. As references, roll-refined dark chocolates with a fat content of 33% and 36% were prepared by means of conventional processing (Section 4.3.2.2) which had a production capacity of 5 kg. Two different cocoa sources, namely cocoa mass (fat content of 53.5%; moisture content of

1.5%; D(v,0.9) of 22 µm) and cocoa powder (fat content of 11.0%; moisture content of 2.4%; D(v,0.9) of 30 µm) were used separately as ingredient. The basic formulation used for the chocolate production can be seen in Table 4.1, and the comparison of the stages in the alternative and conventional processing in Table 4.2.

Table 4.1. Basic formulation of dark chocolate produced by means of alternative processing with total fat of 36% and conventional processing (references) with total fat of 36% and 33%

Samples (total fat content of 36%)

Ingredient	Alternative Processing	
	Cocoa Mass Chocolate	Cocoa Powder Chocolate
	Mass (%wt)	Mass (%wt)
Chocolate 0.4% lecithin		
Sugar	48.00	48.00
Cocoa mass	34.42	-
Cocoa powder	-	17.98
Cocoa butter	17.18	33.62
Lecithin	0.40%	0.40
Chocolate 0.3% lecithin		
Sugar	48.00	48.00
Cocoa mass	34.42	-
Cocoa powder	-	17.98
Cocoa butter	17.28	33.72
Lecithin	0.30	0.30
Chocolate 0.2% lecithin		
Sugar	48.00	48.00
Cocoa mass	34.42	-
Cocoa powder	-	17.98
Cocoa butter	17.38	33.82
Lecithin	0.20	0.20

References 1 (total fat content of 36%)

Ingredient	Conventional Processing	
	Cocoa Mass Chocolate	Cocoa Powder Chocolate
	Mass (%wt)	Mass (%wt)
Chocolate 0.4% lecithin		
Sugar	48.00	48.00
Cocoa mass	34.42	-
Cocoa powder	-	17.98
Cocoa butter	17.18	33.62
Lecithin	0.40	0.40
Chocolate 0.3% lecithin		
Sugar	48.00	48.00
Cocoa mass	34.42	-
Cocoa powder	-	17.98
Cocoa butter	17.28	33.72
Lecithin	0.30	0.30

Chocolate 0.2% lecithin		
Sugar	48.00	48.00
Cocoa mass	34.42	-
Cocoa powder	-	17.98
Cocoa butter	17.38	33.82
Lecithin	0.20	0.20

References 2 (total fat content of 33%)

Ingredient	Conventional Processing	
	Cocoa Mass Chocolate	Cocoa Powder Chocolate
	Mass (%wt)	Mass (%wt)
Chocolate 0.4% lecithin		
Sugar	48.00	48.00
Cocoa mass	40.88	-
Cocoa powder	-	21.35
Cocoa butter	10.72	30.25
Lecithin	0.40	0.40
Chocolate 0.3% lecithin		
Sugar	48.00	48.00
Cocoa mass	40.88	-
Cocoa powder	-	21.35
Cocoa butter	10.82	30.35
Lecithin	0.30	0.30
Chocolate 0.2% lecithin		
Sugar	48.00	48.00
Cocoa mass	40.88	-
Cocoa powder	-	21.35
Cocoa butter	10.92	30.45
Lecithin	0.20	0.20

Key experimental variables in this research were lecithin concentration, ball size and milling time. A 3 x 2 x 3 factorial experimental design was used with lecithin concentrations of 0.2%, 0.3%, and 0.4%; ball sizes of 6.5 mm and 9.5 mm; and milling times of 15 min, 30 min and 45 min. The lecithin concentrations used for the reference chocolates were also 0.2%, 0.3%, and 0.4%. The parameters used to evaluate this experiment were moisture content, particle size distribution and rheological behaviour.

In order to easily recognise the samples, a sample code system was made in this study. In the alternative processing method, 15, 30 and 45 indicated the 15 min, 30 min, 45 min milling time, respectively; S and B indicated the small balls and big balls, respectively; 2, 3 and 4 indicated the 0.2%, 0.3%, and 0,4% lecithin concentration, respectively; CM and CP indicated that cocoa mass and cocoa powder were used as cocoa source, respectively. In the conventional processing, Ref indicated the reference chocolate; 33% and 36% indicated that the chocolate contains 33% and 36% fat, respectively.

4.3.2.1 Combination of a ball mill and a Stephan mixer equipped with a vacuum pump as an alternative chocolate production system

Based on the necessity of small scale chocolate production system which should be capable of producing homogeneous chocolate suspension with good flowability, a Stephan mixer equipped with a vacuum pump, as a liquefier device, was used in combination with a ball mill as a refiner. This production system, termed as alternative processing, consisted of three steps, namely 1) mixing and coarse "dry conching" with Stephan Universal Machine UMC 5 (Stephan food service equipment GmbH, Hameln, Germany), 2) refining/milling and wet "conching" using a ball mill with numerous metal balls weighing 25kg in total (Duyvis Wiener, Koogaan de Zaan, Netherlands) and 3) liquefaction and additional wet "conching" in the Stephan mixer.

Prior to the refining process the ingredients were mixed and "conched" together in the Stephan mixer. This process was divided in 2 steps. The first mixing step was carried out at a temperature of 70^{o}C and a blade speed of 750 rpm for 60 min. In this step, the fat content of the mixture was adjusted to 28% fat. A vacuum pump was used during the last 10 min at a vacuum pressure of -1 bar (gauge pressure) to reduce the moisture and volatile acidity. The second mixing step was performed at a temperature of 50^{o}C and a blade speed of 1500 rpm for 30 min. Prior to the second mixing, the remainder of the cocoa butter and 0.2% lecithin were added. Subsequently, refining of the mixture was performed in the ball mill for 15, 30, or 45 min (depending on the set up) at a temperature of 50^{o}C and a maximum speed of 240 rpm.

Once the ball milling process was finished, the collection of the chocolate mass was performed by opening the valve underneath the ball mill tank/cylinder. To be able to collect the chocolate mass, the stirring process at a speed of 120 rpm, was used. Afterwards, liquefaction process was needed to improve the rheological behaviour. Prior to the liquefaction step, the remainder of the lecithin was added to create chocolates with 0.2%, 0.3%, and 0.4% lecithin concentration. In order to manage the fat content of the chocolate to remain at 36% (sample and reference) or 33% (reference), lecithin was added complementary to the cocoa butter.

The liquefaction step was conducted in the Stephan mixer at a temperature of 50^{o}C and a blade speed of 1500 rpm for 15 min. Liquid chocolates were then sampled in sealed plastic containers at ambient temperature and used for particle size distribution (PSD), rheological behaviour and moisture content analyses. All measurements were done in triplicate.

4.3.2.2 Conventional chocolate production system

As references, dark chocolates formulated with both cocoa mass and cocoa powder were conventionally produced using Vema mixer BM 30/20 (NV Machinery Verhoest, Izegem, Belgium), Exakt 80S 3-roll refiner (Exakt Apparatebau, Norderstedt, Germany) and Bühler Elk'Olino conche (Richard Frisse GmbH, Bad Salzuflen, Germany) with a production capacity of 5 kg. The processing method employed was similar to the one described by Tran et al. (2015b) with a temperature modification in the conching stage, which was set at 70^{o}C instead of 80^{o}C. In the described method, conching process consisted of two phases, namely dry and wet phase. The dry phase was conducted at a fat content of 28%, herein, mixing was done at 60°C and 1200 rpm for 2 h, while shearing was done at 80°C and 1200 rpm for 4 h. Prior to the wet phase, the remainder of cocoa butter and lecithin were added. In this step, mixing followed by shearing was conducted at 45°C and 2400 rpm for 30 min.

Table 4.2. Comparison of the machine employed in each chocolate processing step in the alternative and conventional processing

Processing Method	Chocolate production steps			
	Mixing	Refining	Flavour, texture and flow development	Liquefaction
Alternative processing	Stephan mixer (low shear)	Ball mill-refiner	Stephan mixer (low shear)	Stephan mixer (high shear)
Conventional processing	Planetary mixer	Roll-refiner	Conche	Conche

4.3.3 ANALYTICAL METHODS

The moisture content of the dark chocolates was determined by means of Karl-Fischer titration method. The analysis was performed using a 719 Titrino (Metrohm, Switzerland) with Hydranal solvent (Riedel de Haen, 34812) and Hydranal titrant 5 (Riedel de Haen, 34801). Prior to the moisture analysis, chocolate was melted in an oven at 55 ^{o}C for at least an hour.

The free cocoa butter (fat) content of the chocolates was determined by dissolving the free cocoa butter in the chocolates with petroleum ether (Fisher Scientific, UK). Petroleum ether was added to 10 g of molten chocolate to have a final volume of 40 ml and was then mixed using a vortex. Afterwards, the mixture was centrifuged using Sigma Centrifuge 4K15 (Sigma Laborzentrifugen GmbH, Germany) for 10 min at 9000 rpm. The supernatant was then separated by pouring it into a bulb flask. The precipitate was then exposed to the same procedure for 2 more times. Afterwards, the total supernatant was put in a vacuum rotary evaporator for 10 min at 60 ^{o}C to evaporate the solvent. Before weighing the free cocoa butter, the bulb flask was put in the oven at 105 ^{o}C for 2 hours to further evaporate the solvent.

The particle size distribution (PSD) of the dark chocolates was evaluated by Laser diffraction method using a Malvern Mastersizer S Long Bench (Malvern Instruments Ltd, Worcestershire, UK) equipped with a 300 RF lens to measure particles in a range of 0.05-900 µm. Presentation setting of 30HE (1.6100, 0.1000 in 1.4000) was used in this measurement. The first, second and third value shows the relative refractive index of the particle, the particle adsorption and the refractive index of the dispersant, respectively. Additional devices consisting of a vessel and a pump to circulate the isopropanol suspension were installed. Approximately 0.5 g of chocolate was diluted in 10 ml of isopropanol and heated in an oven at 55^{o}C for no less than one hour. The diluted chocolate was then shaken vigorously prior to the measurement. The dissolved chocolate was then added dropwise into isopropanol at room temperature until reaching an obscuration between 15-30 %. D(v,0.9), D(v,0.5), D(v,0.1), D(4,3) and D(3,2) and span, representing respectively a percentile of 90%, 50% and 10%, volume-weighed mean, Sauter diameter and distribution width, respectively, were derived from the result of the measurement.

An AR2000 rheometer (TA instruments) equipped with a concentric cylinder geometry (cylinder: 42.00 mm, rotor outer radius: 14.00 mm, stator inner radius: 15.00 mm, geometry gap: 5920 µm) was used to determine the chocolate flow parameters. Measurements were performed based on the International Confectionery Association (ICA) official method 46 (ICA, 2000). The chocolates were heated at 52 ^{o}C in the oven for 1 hour prior to measurement. Approximately 20 g of chocolate was used per measurement. Samples were pre-sheared at 5 s^{-1} at 40 ^{o}C for 15 min prior to the measurement. The shear stress was measured by increasing the shear rate step-wise from 2 s^{-1} to 50 s^{-1} (ramp up) for 16 s / step, holding at 50 s^{-1} for 60 s, and then decreasing the shear rate from 50 s^{-1} to 2 s^{-1} (ramp down) for 16 s / step. The data of the upward flow curve were then fitted to the Casson model to derive the Casson yield stress (σ_{CA}) and Casson viscosity (η_{CA}). Thixotropy was obtained by determining the difference between the ramp up and ramp down shear stress at 5 s^{-1}. The measurement was conducted in triplicate.

The microstructure of chocolate was observed using a Leica DM2500 microscope (Wetzlar, Germany) under normal and polarised light to observe the presence of agglomerates and sugar crystal arrangement in the chocolate, respectively. For sugar crystal visualisation, a drop of molten chocolate previously heated at 55^{o}C for 1 h was put on a glass slide. For the agglomerates visualisation, approximately 0.5 g of chocolate was diluted in 10 ml of isopropanol and heated in an oven at 55 ^{o}C for 1 h. A cover slip was then placed over the sample and adjusted to ensure that the sample thickness was uniform. To stabilise the image of the samples during observation, the temperature of the glass slide support was set at 50 ^{o}C on a hot stage connected to a Linkam T95 System Controller (Linkam Scientific Instrument Ltd, Surrey, UK).

4.3.4 DATA ANALYSIS

Data analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). Moisture level, free cocoa butter content, PSD, and flow behaviour parameters were subjected to variance analysis (ANOVA) with a 5% significance level. Testing for homogeneity of variances was performed with Levene Test. When the conditions for homogeneity of variances were fulfilled, Tukey test was used to determine the differences among samples. In case variances were not homogeneous, Games–Howell test was performed. Three-way analysis of variance (ANOVA) were used to determine the effects of ball size, milling time, lecithin concentration, and their interactions on the PSD parameters, Casson yield value, Casson viscosity and thixotropy. Principal component analysis (PCA) was used to visualise the relationships between chocolates samples, moisture, PSD and flow behaviour parameters.

4.4 RESULTS AND DISCUSSION

4.4.1 Interconnections between lecithin concentration, ball size and milling time in the PSD and the rheological behaviour of chocolates produced by alternative processing

The interconnections between lecithin concentration, ball size and milling time in determining the rheological behaviour of chocolates produced by combination of ball mill and Stephan mixer as a liquefier device were investigated using multivariate and principal component analysis. Aside from this, it was also imperative to find out the interconnections between lecithin concentration, ball size, and milling time in defining the particle size distribution, which has been reported to have a direct relationship with the rheological behaviour of chocolate (Afoakwa, 2010; Beckett, 2009). Knowing the experimental variables and their interactions affecting the PSD and the rheological behaviour of chocolate is very important for the development of this production system.

Table 4.3. Interconnections between lecithin concentration, ball size and milling time in the particle size distribution and rheological behaviour of chocolates produced by alternative processing

Cocoa Mass Chocolate

Experimental Variables	Particle size distribution						Flow behaviour		
	Distribution percentiles (µm)			Derived diameter (µm)		Span (-)	Casson yield value (Pa)	Casson viscosity (Pa.s)	Thixotropy (Pa)
	D(v,0.9)	D(v,0.5)	D(v,0.1)	D(4,3)	D(3,2)				
Time (T)	*	*	*	*	*	*	*	*	*
Lecithin (L)							*	*	*
Ball size (B)	*	*	*	*	*		*	*	*
Interaction T x L							*	*	*
Interaction T x B	*			*		*	*	*	*
Interaction L x B							*	*	

*) Significant at p<0.05

Cocoa Powder Chocolate

Experimental Variables	Particle size distribution						Flow behaviour		
	Distribution percentiles (µm)			Derived diameter (µm)		Span	Casson yield value (Pa)	Casson viscosity (Pa.s)	Thixotropy (Pa)
	D(v,0.9)	D(v,0.5)	D(v,0.1)	D(4,3)	D(3,2)				
Time (T)	*	*	*	*	*	*	*	*	*
Lecithin (L)							*	*	*
Ball size (B)	*	*	*	*	*		*	*	*
Interaction T x L							*	*	*
Interaction T x B	*	*	*	*	*		*		*
Interaction L x B							*		

*) Significant at p<0.05

It can be seen in Table 4.3 that the PSD of the chocolates produced by means of alternative processing either formulated with cocoa mass or cocoa powder was significantly influenced by milling time and ball size ($p<0.05$) but not by the lecithin concentration. Conversely, the span was only significantly influenced by milling time. All the PSD parameters, except span, of chocolates formulated with cocoa powder were also influenced by the interaction of ball size and milling time, whereas only D(v,0.9), D(4,3) and span of the chocolates formulated with cocoa mass were influenced by this interaction. Furthermore, the milling time, lecithin concentration and ball size significantly influenced ($p<0.05$) Casson yield value, Casson viscosity and thixotropy of the chocolates either formulated with cocoa mass or cocoa powder. All the binary interactions ($p<0.05$) between the three processing parameters significantly affected the Casson yield value and viscosity of the chocolates formulated with cocoa mass and the Casson yield value of the chocolates formulated with cocoa powder. While the Casson viscosity of the chocolates formulated with cocoa powder was only influenced by the interaction between the milling time and the lecithin concentration. Furthermore, it can also be seen that all the experimental variables and their binary interactions influenced thixotropy, with the exception of the interaction between lecithin concentration and ball size.

As mentioned above, lecithin concentration and its interactions with the studied processing variables did not influence the PSD of the chocolates. This phenomenon showed that during the subsequent liquefaction process, where the remainder of the lecithin was added, further particle size reduction did not occur. As previously mentioned in Section 4.3.2.1, during the ball milling process where the particle size reduction occurred, all the chocolates contained the same amount of lecithin.

The PCA plots in Figure 4.1 and Figure 4.2 give a general overview of the relationship between the PSD and the flow parameters of the chocolates formulated with cocoa mass and cocoa powder, respectively. It can be observed that based on the PCA score plot, regardless of the cocoa source, the chocolates produced by means of alternative processing are located close to each other and no clear clusters were observed. However, the chocolates produced by means of alternative processing can be separately clustered from the ones produced by means of conventional processing.

From the PCA data analysis of the chocolates formulated with cocoa mass (Figure 4.1), it can be seen that the first 2 factors explained more than 72% of the variance, namely PC1 : 41.7% and PC2 : 31.2%. In general, the difference between alternative and conventional processing can be observed along PC2, while the milling time effect in the alternative processing can be identified through PC1. Chocolates produced by means of alternative processing had higher values than chocolates produced by conventional processing which can be observed in PC 2 (Figure 4.1A). This indicated that chocolates produced by means of alternative processing exhibited higher Casson yield and thixotropy values as well as slightly higher Casson plastic viscosities compared to the ones produced by conventional processing (Figure 4.1B). The 15-min milling time, in contrast to the 45-min milling time, of the chocolates produced by alternative processing can be characterised by high positive values in PC1 which indicates a lower Casson yield and Casson Viscosity values. However, as seen in Figure 4.1, lack of trends were observed for the impact of lecithin concentration and ball size on the rheological properties. This phenomenon may be attributed, to some extent, to the formation of reverse micelles dispersed in cocoa butter and/or the formation of self-association of lecithin possibly as multilayers around sugar in some chocolates (Afoakwa et al., 2007; Beckett, 2009).

The PCA data analysis of the chocolates formulated with cocoa powder is shown in Figure 4.2. It can be seen that the first 2 factors explained more than 79% of the variance, namely PC1 : 63.3% and PC2 : 16.3%. It can be observed in PC 1 that chocolates produced by means of alternative processing had lower values than chocolates produced by conventional processing (Figure 4.2A). This indicated that chocolates produced by means of alternative processing exhibited higher Casson yield values as well as slightly higher Casson viscosities and thixotropy compared to the ones produced by conventional processing (Figure 4.2B). The 15-min milling time, in contrast to the 45-min milling time, of the chocolates produced by alternative processing was characterised by high positive values in PC2 indicating a lower Casson yield value and Casson Viscosity. Similar to the chocolate formulated with cocoa mass, the impact of lecithin concentration and ball size on the rheological behaviour could also not be clearly seen.

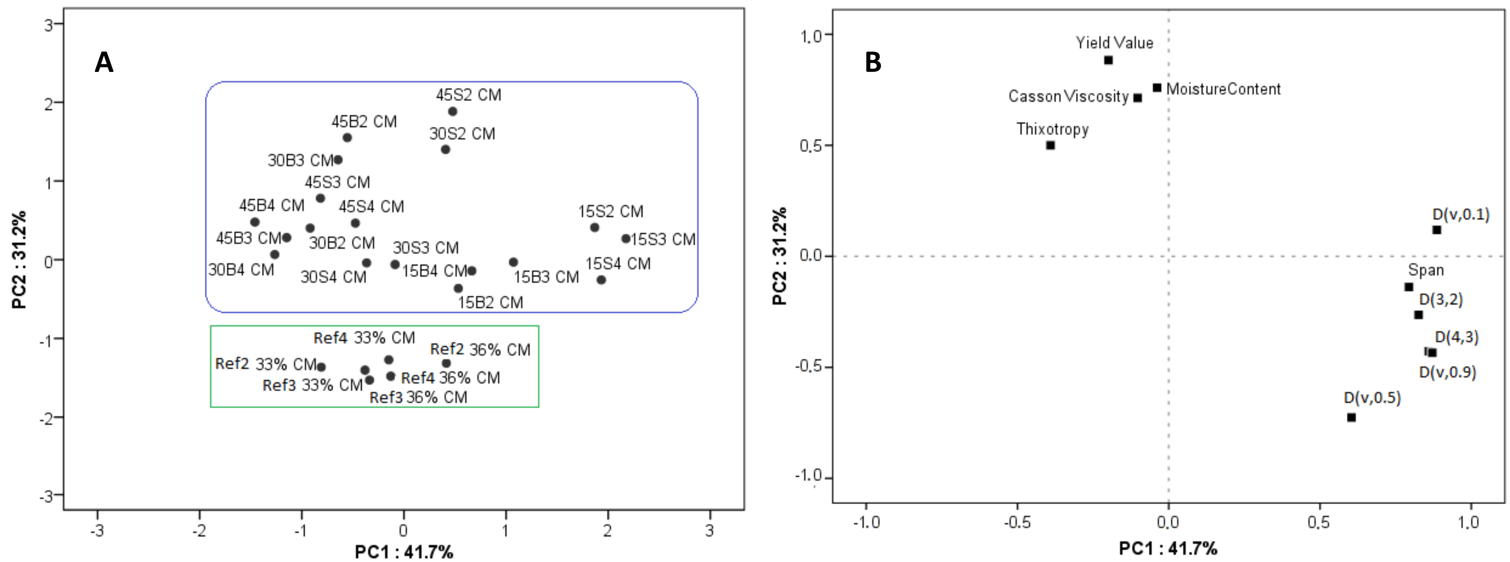

Figure 4.1. PCA score plot (A) of chocolates formulated with cocoa mass produced using different lecithin concentrations, ball sizes and milling times. PCA loading plot (B) of moisture, PSD and flow parameters of chocolates formulated with cocoa mass.

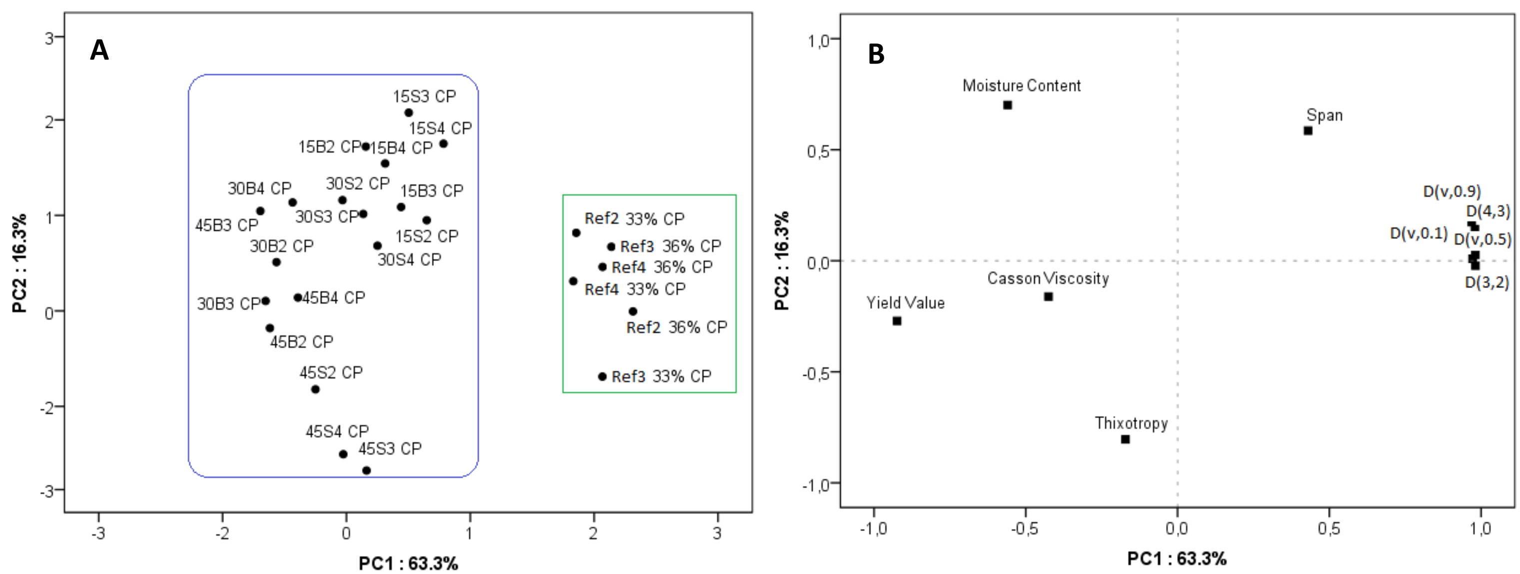

Figure 4.2. PCA score plot (A) of chocolates formulated with cocoa powder produced using different lecithin concentrations, ball sizes and milling times. PCA loading plot (B) of moisture, PSD and flow parameters of chocolates formulated with cocoa powder.

4.4.2 Feasibility of a ball mill combined with Stephan mixer as a liquefier device for alternative chocolate production system

The performance of a chocolate production system can be evaluated by assessing the characteristics of the chocolate manufactured. The most important capabilities that should be inherent to a chocolate production system are the ability in evaporating moisture and/or undesirable volatiles, reducing the sizes of the solid particles, creating homogeneous mixtures, and developing desired rheological characteristics (Saputro et al., 2016a; Saputro et al., 2016b). Those abilities can be assessed by measuring respectively moisture content, particle size distribution and flow properties of the chocolates. It is well known that with the same fat level and lecithin concentration, particle size distribution together with moisture content highly influence not only the flow behaviour of molten chocolate, but also other quality attributes, such as colour, hardness, aroma profile *etc.* (Afoakwa, 2010; Saputro et al., 2016a; Stortz and Marangoni, 2011). Therefore, by measuring the moisture level, particle size distribution and rheological behaviour of chocolate, the other quality attributes, to some extent, can be estimated.

4.4.2.1 Moisture content

The presence of moisture in chocolate matrix either dissolves sugar particles or creates sticky patches on the surfaces of the sugar, thus increasing viscosity (Beckett, 2008). Therefore, it is very imperative to reduce the moisture level to less than 1% (Afoakwa, 2010). Table 4.4 shows that, irrespective of the cocoa source and the processing method, all chocolates had comparable moisture content ranging between 0.58% and 0.69**%.** The moisture content of the chocolate mixture before processing of both cocoa mass and cocoa powder chocolate (0.62% and 0.64% respectively) which were already very low may explain this. Moreover, this phenomenon showed that the alternative processing had comparable capability to the conventional one of maintaining the moisture content below 1%. Glicerina et al. (2013) reported that during refining process, the newly formed sugar surfaces could absorb the humidity from the surrounding, which did not occur in this study. From the results obtained, regardless of milling time and ball size, the lecithin concentration did not affect the moisture content of the chocolates (data not shown).

Table 4.4. Moisture and free fat content of dark chocolates formulated with cocoa mass and cocoa powder produced by alternative and conventional processing

Cocoa Mass Chocolate	Moisture (%)	Free fat content (%)
chocolate mixture (prior to processing)	0.62 ± 0.00[a]	-
15S CM	0.60 ± 0.00[a]	35.8 ± 0.4[b]
30S CM	0.65 ± 0.11[a]	35.8 ± 0.4[b]
45S CM	0.69 ± 0.11[a]	36.0 ± 0.2[b]
15B CM	0.60 ± 0.12[a]	35.9 ± 0.7[b]
30B CM	0.67 ± 0.10[a]	35.7 ± 0.2[b]
45B CM	0.61 ± 0.00[a]	35.8 ± 0.4[b]
Ref 33% CM	0.56 ± 0.00[a]	32.7 ± 0.2[a]
Ref 36% CM	0.59 ± 0.00[a]	35.8 ± 0.2[b]

Cocoa Powder Chocolate	Moisture (%)	Free fat content (%)
chocolate mixture (prior to processing)	0.64 ± 0.0[a]	-
15S CP	0.67 ± 0.00[a]	35.9 ± 0.5[b]
30S CP	0.67 ± 0.00[a]	35.8 ± 0.1[b]
45S CP	0.58 ± 0.10[a]	35.9 ± 0.3[b]
15B CP	0.67 ± 0.00[a]	36.0 ± 0.2[b]
30B CP	0.66 ± 0.00[a]	35.9 ± 0.3[b]
45B CP	0.67 ± 0.10[a]	36.0 ± 0.3[b]
Ref 33% CP	0.59 ± 0.00[a]	32.6 ± 0.0[a]
Ref 36% CP	0.59 ± 0.00[a]	35.6 ± 0.2[b]

values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

4.4.2.2 Free cocoa butter content

Free cocoa butter content can be described as cocoa butter that is not trapped in the cocoa particles. Its presence in the chocolate matrix is associated with the addition of cocoa butter as one of the chocolate ingredients and also with the presence of the free cocoa butter in cocoa mass/liquor. During chocolate production, additional cocoa butter can also be released from the cocoa particles, depending on the temperature, time and shear applied (van der Vaart et al., 2013). The more the free cocoa butter present in the chocolate suspension, the lower the Casson yield value and Casson plastic viscosity will be. Therefore, the investigation of the free cocoa butter content is also very important.

It can be seen in Table 4.4 that all the chocolates had comparable free fat content. Chocolates with total fat content of 36% contained almost 36% of free fat, while chocolates with total fat content of 33% contained almost 33% of free fat. This phenomenon showed that during chocolate production, almost all cocoa butter trapped in the cocoa particles, regardless of the cocoa source, was released. Moreover, this phenomenon indicated that even though ball mill processing does not have similar shearing system as the one in the conventional processing, the friction among the balls as well as the friction between the balls and the wall of the ball mill tank triggered the release of additional cocoa butter.

4.4.2.3 Particle size distribution

PSD is a key determinant of the chocolate flow properties which does influence not only consumer perception, but also handling properties (Afoakwa, 2010; Servais et al., 2002). Aside from this, particle size distribution relates to the fineness of chocolate, with particle size higher than 30 µm, chocolate is perceived as 'gritty', while chocolate with particle size smaller than 20 µm has a creamier taste and texture (Afoakwa, 2010).

The PSD parameters showed variations in the values of D(v,0.9), D(v,0.5), D(v,0.1), D(4,3) and D(3,2). Nevertheless, in general, it can be seen in Table 4.5 that the D(v,0.9) value is directly proportional to the D(v,0.5), D(v,0.1), Sauter mean diameter D(3,2) and volume-weighed mean D(4,3). In this work D(v,0.9) was used to represent the fineness of the solid particles as it has been reported to correlate fairly well with what people actually taste (Beckett, 2008; van der Vaart et al., 2013).

It can be seen in Table 4.5 that the chocolates formulated either with cocoa powder or cocoa mass produced with the same experimental variables exhibited quite similar D(v,0.9). The differences in D(v,0.9) value between them were only 1-2 µm, with the exception in the chocolates produced using small balls with 15 min milling time which had a D(v,0.9) difference of around 4 µm. Furthermore, it can also be seen that the chocolates formulated with cocoa mass produced by alternative processing had only slightly lower D(v,0.9) values than the reference chocolates formulated with cocoa mass. However, the chocolates formulated with cocoa powder produced by alternative processing exhibited significantly lower ($p<0.05$) D(v,0.9) values than the reference chocolates. Since the production of the chocolates used the same protocols, the difference in the size of the solid particles of the chocolates produced by conventional processing may be due to the different breakdown behaviour of cocoa mass and cocoa powder. Apart from that, the fact that cocoa powder had higher PSD than cocoa mass should also not be neglected.

Table 4.5. Particle size distribution of dark chocolates formulated with cocoa mass and cocoa powder produced by means of alternative and conventional processing

Cocoa Mass Chocolate	D(v,0.9)µm	D(v,0.5) µm	D(v,0.1) µm	D(4,3) µm	D(3,2) µm	Span (-)
15S CM	26.2 ± 0.3^{e}	6.6 ± 0.1^{e}	1.4 ± 0.0^{d}	10.9 ± 0.2^{f}	2.6 ± 0.1^{d}	3.8 ± 0.0^{e}
30S CM	18.1 ± 0.1^{b}	5.8 ± 0.1^{cd}	1.3 ± 0.0^{cd}	8.2 ± 0.1^{c}	2.4 ± 0.1^{abcd}	2.9 ± 0.0^{bc}
45S CM	16.4 ± 0.5^{a}	5.6 ± 0.1^{bc}	1.3 ± 0.1^{bcd}	7.6 ± 0.2^{ab}	2.4 ± 0.1^{abcd}	2.7 ± 0.0^{a}
15B CM	22.9 ± 0.3^{d}	6.1 ± 0.1^{d}	1.3 ± 0.1^{bcd}	9.9 ± 0.1^{e}	2.4 ± 0.1^{bcd}	3.6 ± 0.0^{d}
30B CM	17.3 ± 0.2^{b}	5.4 ± 0.1^{ab}	1.2 ± 0.1^{a}	7.8 ± 0.1^{b}	2.2 ± 0.1^{a}	3.0 ± 0.1^{c}
45B CM	16.0 ± 0.1^{a}	5.3 ± 0.1^{a}	1.2 ± 0.0^{ab}	7.4 ± 0.1^{a}	2.2 ± 0.1^{ab}	2.8 ± 0.1^{b}
Ref 33% CM	20.6 ± 0.3^{c}	6.5 ± 0.1^{e}	1.2 ± 0.1^{abc}	9.0 ± 0.1^{db}	2.3 ± 0.1^{ac}	3.0 ± 0.1^{c}
Ref 36% CM	21.1 ± 0.4^{c}	6.8 ± 0.2^{e}	1.3 ± 0.1^{abc}	9.3 ± 0.2^{d}	2.6 ± 0.1^{cd}	2.9 ± 0.0^{bc}

Cocoa Powder Chocolate	D(v,0.9)µm	D(v,0.5) µm	D(v,0.1) µm	D(4,3) µm	D(3,2) µm	Span (-)
15S CP	21.6 ± 0.1^{e}	6.2 ± 0.0^{c}	1.3 ± 0.0^{d}	9.3 ± 0.1^{e}	2.4 ± 0.0^{d}	3.3 ± 0.0^{d}
30S CP	17.9 ± 0.3^{c}	5.7 ± 0.1^{b}	1.2 ± 0.0^{cd}	8.0 ± 0.1^{c}	2.3 ± 0.0^{cd}	3.0 ± 0.1^{c}
45S CP	15.7 ± 0.1^{b}	5.3 ± 0.1^{a}	1.1 ± 0.0^{bc}	7.2 ± 0.0^{b}	2.2 ± 0.1^{bc}	2.8 ± 0.0^{ab}
15B CP	20.4 ± 0.2^{d}	5.9 ± 0.1^{b}	1.2 ± 0.0^{bcd}	8.9 ± 0.1^{d}	2.3 ± 0.1^{cd}	3.3 ± 0.0^{d}
30B CP	15.7 ± 0.1^{b}	5.1 ± 0.1^{a}	1.0 ± 0.0^{a}	7.1 ± 0.1^{b}	2.0 ± 0.1^{a}	2.9 ± 0.0^{bc}
45B CP	14.6 ± 0.2^{a}	5.0 ± 0.1^{a}	1.1 ± 0.0^{ab}	6.8 ± 0.1^{a}	2.0 ± 0.1^{ab}	2.7 ± 0.0^{a}
Ref 33% CP	27.5 ± 0.2^{g}	8.2 ± 0.1^{d}	1.5 ± 0.0^{e}	11.7 ± 0.1^{f}	2.9 ± 0.1^{e}	3.2 ± 0.0^{d}
Ref 36% CP	26.8 ± 0.1^{f}	8.6 ± 0.2^{e}	1.5 ± 0.1^{f}	11.8 ± 0.1^{f}	2.9 ± 0.1^{f}	3.0 ± 0.1^{c}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

In general, regardless of the cocoa source, with the same milling time, the chocolates produced using big balls had significantly lower ($p<0.05$) D(v,0.9) values than the chocolates produced with small balls. This difference may be due to a higher force generated by the big balls during milling process, resulting in smaller sized solid particles. Regardless of the cocoa source and the ball size, the chocolates produced with milling time of 30 min had significantly lower D(v,0.9) values than the chocolates produced with milling time of 15 min. However, the chocolates milled in 45 min only had slightly lower D(v,0.9) than the chocolates milled in 30 min, showing that after 30 min of milling, the reduction of particle size was not significant anymore. Moreover, span, as an indicator for the width of the PSD, decreased as the milling time was increased. Based on this observation, in order to save more time and energy, 30 min milling time was recommended.

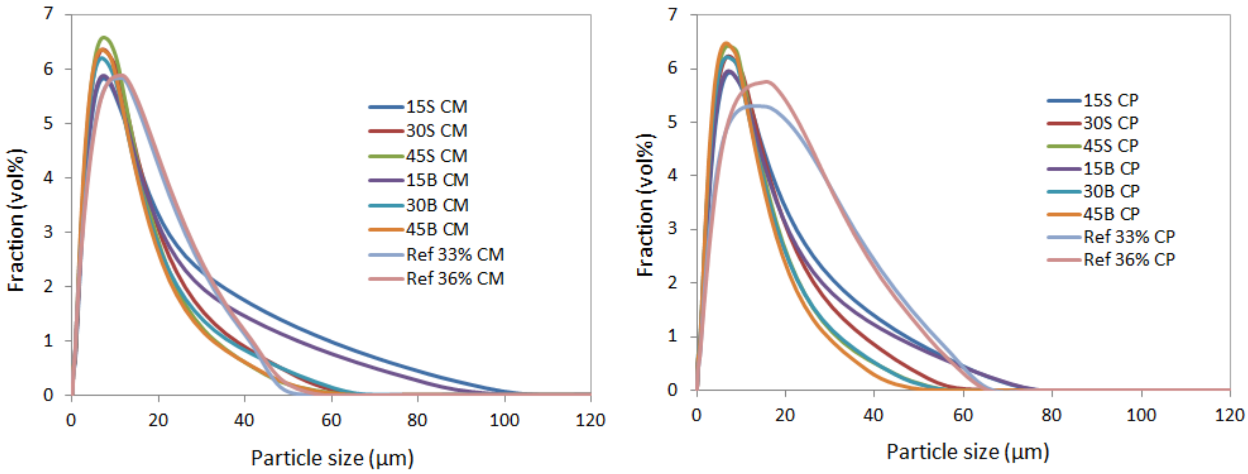

Figure 4.3. PSD curve of chocolates formulated with cocoa mass and cocoa powder produced by means of alternative and conventional processing

As can be seen in Figure 4.3, the chocolates produced by means of alternative processing exhibited a different curve shape as compared to the ones produced by conventional processing. Even though both types of chocolate had similar curve shapes, the chocolates produced by alternative processing tended to have substantial "tail", which was not observed in the chocolates produced by conventional processing. The presence of the "tail" showed the presence of a group of coarser particles in the chocolate suspension. This phenomenon can be explained by the fact that the collection of chocolate mass from the batch-type ball mill tank relied on the gravitational power. During this process, the metal balls were also stirred, resulting in further particle size reduction. Therefore, the earlier the chocolate mass collected, the bigger the PSD of the chocolate was. The presence of a group of coarser particle might influence the sensory perception. Aside from this, its presence might also influence the packing efficiency of solid particles which in turn might influence the rheological behaviour. The impact of packing efficiency on the rheological behaviour of chocolate is discussed in section 4.4.2.4.

For better chocolate application, in order to have the same type of PSD curve as the chocolates produced by means of conventional processing which contain more uniform particle sizes of solids, the chocolates produced by alternative processing can be partially mixed with the chocolates produced by conventional processing. Nevertheless, this approach has to be further investigated. Aside from this, the necessity for the conventional processing machines requires a higher capital investment which is mostly not feasible for small scale chocolate production.

4.4.2.4 Rheological behaviour

Molten chocolate exhibits a non-ideal plastic behaviour, more particularly shear-thinning behaviour occurs as soon as yield value has been exceeded (Afoakwa, 2010; Aidoo, 2015). The most widely used model to fit this behaviour is the Casson model. From this model, Casson yield stress, which can be described as the stress required to start the flow, and Casson viscosity, which can be described as the internal friction during the flow, are derived. Flow behaviour of chocolate is known to be influenced by PSD, lecithin, fat and moisture content (Afoakwa, 2010; Beckett, 2009). However, in this study, PSD and lecithin became the major factors because all the chocolates had a comparable moisture, free fat content (Table 4.4) and also total fat content.

Table 4. 6. Flow parameters of dark chocolates formulated with cocoa mass and cocoa powder produced by means of alternative and conventional processing

Cocoa Mass Chocolate	Casson yield value (Pa)	Casson viscosity (Pa.s)	Thixotropy (Pa)
15S2 CM	13.47 ± 0.25^{g}	3.40 ± 0.06^{j}	0.71 ± 0.24^{abcd}
15S3 CM	11.70 ± 0.15^{d}	2.53 ± 0.11^{efg}	1.16 ± 0.51^{bcdef}
15S4 CM	11.02 ± 0.43^{c}	2.24 ± 0.04^{d}	0.62 ± 0.34^{abcd}
30S2 CM	16.42 ± 0.10^{ij}	4.92 ± 0.03^{m}	0.50 ± 0.48^{abcd}
30S3 CM	14.19 ± 0.13^{h}	2.96 ± 0.03^{h}	0.73 ± 0.13^{abcd}
30S4 CM	13.42 ± 0.18^{fg}	2.43 ± 0.07^{e}	0.52 ± 0.08^{abcd}
45S2 CM	17.44 ± 0.27^{lm}	6.44 ± 0.11^{n}	0.70 ± 0.33^{abcd}
45S3 CM	17.08 ± 0.07^{kl}	3.35 ± 0.05^{j}	1.78 ± 0.18^{f}
45S4 CM	16.06 ± 0.13^{ij}	2.64 ± 0.04^{fg}	1.58 ± 0.29^{ef}
15B2 CM	16.59 ± 0.11^{jk}	3.13 ± 0.04^{hi}	0.17 ± 0.04^{a}
15B3 CM	12.80 ± 0.06^{ef}	2.24 ± 0.02^{d}	0.22 ± 0.07^{a}
15B4 CM	12.42 ± 0.08^{e}	1.96 ± 0.02^{c}	0.35 ± 0.24^{ab}
30B2 CM	20.17 ± 0.12^{p}	4.30 ± 0.04^{l}	0.68 ± 0.36^{abcd}
30B3 CM	18.00 ± 0.10^{mn}	3.14 ± 0.11^{i}	2.61 ± 0.51^{g}
30B4 CM	15.91 ± 0.23^{i}	2.16 ± 0.06^{d}	1.28 ± 0.07^{cdef}
45B2 CM	20.53 ± 0.55^{p}	8.94 ± 0.03^{o}	0.90 ± 0.04^{abcde}
45B3 CM	18.86 ± 0.33^{o}	3.74 ± 0.05^{k}	1.30 ± 0.05^{def}
45B4 CM	18.51 ± 0.10^{no}	2.47 ± 0.03^{ef}	1.93 ± 0.17^{fg}
Ref2 33% CM	4.89 ± 0.04^{b}	3.09 ± 0.01^{hi}	0.33 ± 0.09^{a}
Ref3 33% CM	3.92 ± 0.11^{a}	2.65 ± 0.02^{g}	0.52 ± 0.11^{abcd}
Ref4 33% CM	4.65 ± 0.18^{b}	2.42 ± 0.02^{e}	0.34 ± 0.09^{a}
Ref2 36% CM	3.99 ± 0.09^{a}	1.47 ± 0.02^{b}	0.58 ± 0.07^{abcd}
Ref3 36% CM	3.47 ± 0.06^{a}	1.37 ± 0.02^{ab}	0.39 ± 0.15^{ab}
Ref4 36% CM	3.52 ± 0.03^{a}	1.29 ± 0.05^{a}	0.48 ± 0.06^{abc}

Cocoa Powder Chocolate	Casson yield value (Pa)	Casson viscosity (Pa.s)	Thixotropy (Pa)
15S2 CP	23.93 ± 0.82^{fg}	2.18 ± 0.05^{defgh}	1.83 ± 0.97^{abcd}
15S3 CP	18.52 ± 0.70^{d}	1.92 ± 0.05^{cd}	1.06 ± 0.87^{ab}
15S4 CP	17.70 ± 0.10^{d}	1.61 ± 0.02^{abc}	0.27 ± 0.08^{a}
30S2 CP	25.74 ± 0.56^{h}	2.64 ± 0.04^{ij}	1.07 ± 0.32^{ab}
30S3 CP	22.65 ± 0.32^{ef}	1.99 ± 0.03^{de}	1.65 ± 0.47^{abcd}
30S4 CP	21.80 ± 0.32^{e}	1.90 ± 0.10^{cd}	0.73 ± 0.77^{ab}
45S2 CP	40.12 ± 1.04^{m}	2.58 ± 0.06^{ij}	2.39 ± 0.59^{bcde}
45S3 CP	36.63 ± 0.77^{l}	2.13 ± 0.45^{defg}	4.22 ± 0.40^{ef}
45S4 CP	33.63 ± 0.71^{k}	1.83 ± 0.14^{bcd}	4.53 ± 0.93^{f}
15B2 CP	31.70 ± 0.36^{j}	2.34 ± 0.05^{efghi}	0.51 ± 0.36^{ab}
15B3 CP	25.24 ± 0.14g^{h}	2.05 ± 0.06^{def}	1.66 ± 0.15^{abcd}
15B4 CP	22.60 ± 0.20^{ef}	1.87 ± 0.06^{bcd}	1.32 ± 0.44^{abc}
30B2 CP	38.70 ± 0.53^{m}	2.71 ± 0.09^{ij}	1.05 ± 0.56^{ab}
30B3 CP	34.27 ± 0.24^{k}	2.54 ± 0.24^{hij}	3.27 ± 0.45^{def}
30B4 CP	28.36 ± 0.41^{i}	1.91 ± 0.06^{cd}	0.38 ± 0.31^{a}
45B2 CP	44.61 ± 0.33^{n}	2.82 ± 0.08^{j}	1.63 ± 1.43^{abcd}
45B3 CP	34.83 ± 0.29^{k}	2.13 ± 0.03^{defg}	1.51 ± 0.24^{abcd}
45B4 CP	31.39 ± 0.45^{j}	1.92 ± 0.11^{cd}	1.12 ± 0.57^{ab}
Ref2 33% CP	7.97 ± 0.28^{b}	2.54 ± 0.01^{hij}	0.35 ± 0.09^{a}
Ref3 33% CP	11.62 ± 0.71^{c}	2.51 ± 0.01^{ghij}	3.16 ± 1.01^{cdef}
Ref4 33% CP	13.13 ± 0.48^{c}	2.40 ± 0.02^{fghi}	1.95 ± 0.25^{abcd}
Ref2 36% CP	5.64 ± 0.19^{a}	1.52 ± 0.02^{ab}	1.21 ± 0.36^{ab}
Ref3 36% CP	5.80 ± 0.15^{a}	1.38 ± 0.01^{a}	0.48 ± 0.22^{ab}
Ref4 36% CP	6.73 ± 0.49^{ab}	1.30 ± 0.02^{a}	0.85 ± 0.60^{ab}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

Casson Yield Value

It can be observed in Table 4.6 that at the same lecithin concentration, regardless of the cocoa source, Casson yield values of the chocolates produced by means of alternative processing were higher than the Casson yield values of the chocolates produced by conventional processing. The fact that the chocolates formulated with cocoa mass produced by alternative processing had slightly lower D(v,0.9) values than chocolate produced by conventional processing might be the reason. Similar phenomenon was also observed in the chocolate formulated with cocoa powder. The chocolates produced by alternative processing had significantly lower ($p<0.05$) D(v,0.9) values than the chocolate produced by conventional processing, hence, they had more surface area, resulting in more particle-particle interactions, leading to higher Casson yield values. At low shear stresses, close to the yield value, rheological behaviour is highly influenced by particle-particle interactions (Mongia and Ziegler, 2000), thus Casson yield value will be inversely correlated to the particle size. Aside from this, the different particle surface morphology, especially that of sugar, which is due to different breakage behaviour of particles ground by the ball mill and the roll refiner may also contribute to this phenomenon. According to Servais et al. (2002), surface roughness influences the rheology of suspension. In a ball mill, particles were ground through short contact between sugar/cocoa particles and metal balls and/or wall of the tank, while in a roll refiner particles were refined through a relatively longer contact between solid particles and metal rollers. Middendorf et al. (2015) reported that the difference of flow behaviour of chocolate produced by ball mill and roll refiner can also be attributed to altered surface properties of the sugar particles. Roll refiner tends to create surface of sugar particles with flowing structures, while ball mill tend to create sugar particles with step-like structures (Braun, 2010; Middendorf et al., 2015). This phenomenon can be attributed to the amount of amorphous part formed on the sugar surface (Braun, 2010; Middendorf et al., 2015). Apart from the role of surface properties in determining the Casson yield value, it can be seen in Table 4.6 the reference chocolate which contained 33% fat had a lower Casson yield value than the chocolates with 36% fat content produced by alternative processing, showing the important role of packing volume fraction in determining Casson yield value.

Table 4.6 shows that at the same lecithin concentration, regardless of the cocoa source, the Casson yield value increased as the milling time was increased or as the size of the balls was increased. The fact that the use of longer time and bigger balls resulted in lower particle sizes was the reason for this observation (Table 4.5). It can also be seen that using the same experimental variables, the chocolate formulated with cocoa powder had a higher Casson yield value (Table 4.6) than the chocolate formulated with cocoa mass, which may be attributed to a slightly lower particle size. Moreover, the surface morphology of solid particles, as previously mentioned, which was not investigated in this work might also contribute to this phenomenon.

Generally, the addition of lecithin dramatically reduced the Casson yield value and the Casson viscosity. However, as seen in Table 4.6, an increase in yield value occurred in the reference chocolate with 33% fat (Ref 33%) as the lecithin concentration was increased. This phenomenon was likely associated to the formation of self-association of lecithin as multilayers around sugar particles and/or reverse micelles formation in the continuous phase which reduce the effectiveness of lecithin in reducing Casson yield value (Afoakwa et al., 2007; Beckett, 2009).

The range of Casson yield values for dark chocolate was reported from 4 to 32 Pa (Aeschlimann and Beckett, 2000). Therefore, as can be seen in Table 4.6, most of the Casson yield values of the chocolates are still within this range. In industrial scale, the need for chocolate with certain Casson yield value is based on its application. For biscuit enrobing application for instance, chocolate with low Casson yield value is needed in order to able to easily cover the uneven surface and cracks of the biscuit. Nevertheless, chocolate with relatively high Casson yield value is also still needed to create rugged appearance (Beckett, 2008).

Casson plastic viscosity

Regardless of the cocoa source, at the same lecithin concentration and fat content, the chocolates produced by alternative processing exhibited slightly higher Casson viscosity than the chocolates produced by conventional processing. This observation can be explained by the fact that the D(v,0.9) values of the chocolates produced by alternative processing were lower than that of the chocolates produced by conventional processing and also possible different surface morphology of the particles, as previously discussed. Nevertheless, compared to the reference chocolate with 33% fat, the Casson viscosity of the chocolates produced by alternative processing was comparable, especially the ones formulated with cocoa powder. Similar to the trend of the Casson yield value, regardless of the cocoa source, the Casson viscosity increased as the milling time was increased or as the size of the balls was bigger. The use of longer time and bigger balls resulted in a lower particle size, which was the reason for this observation (Table 4.5).

Casson viscosity, which refers to the internal friction during the flow, is not only influenced by particle size of solid particles, lecithin concentration, moisture level, and fat content but also by maximum volume fraction (Afoakwa, 2010; Beckett, 2009). At the same amount of fat and lecithin, the Casson viscosity of the chocolate suspension can be lowered by optimising volume fraction of the particles. Multimodal or generally broad particle size distribution creates a higher maximum particle volume fraction (Do et al., 2007). In this case, once the small particles fill the voids between the large ones, the amount of fat needed to fill the gaps is reduced (Do et al., 2007). In this condition, an increase in packing fraction, as the number of the particle that can be packed in a given volume, occurs. Hence, more fat will be available for the flow, reducing the Casson viscosity to some extent.

Packing efficiency of the particles relies on the ratio between small and large particles as well as the shape of the particles (Servais et al., 2002). With regard to the ratio between small and large particles, Servais et al. (2002) and Do et al. (2007) stated that, theoretically, in order to have a significant increase in maximum volume fraction, small particles should be at least 7 times smaller than the large ones. With regard to particle shape, Servais et al. (2002) stated that in food suspension, due to the presence of crystalline and amorphous phases combination, the maximum volume fraction observed is in between maximum volume fraction for spherical and non-spherical (cubes) particles. In this experiment, it seemed that packing efficiency of cocoa mass chocolates was lower than that of cocoa powder chocolates. This could be due to the low size ratio of small and large particles and/or due to the occurrence of different shapes of newly formed surface of sugar particles. Sugar mixed with cocoa mass might have different breakdown behaviour during milling as compared to the sugar mixed with cocoa powder, resulting in different amount of amorphous sugar produced. It can be observed in Table 4.6 that the chocolates formulated with cocoa powder and produced by alternative processing had slightly lower D(v,0.9) and Casson viscosity than the chocolates formulated with cocoa mass. However, the Casson viscosity of cocoa powder chocolates theoretically should have been higher than that of cocoa mass chocolates.

The range of Casson viscosity value for dark chocolate was reported to be between 2.1 and 3.9 Pas (Aeschlimann and Beckett, 2000). Therefore, as can be seen in Table 4.6, all the chocolates had acceptable Casson viscosity. It means that the chocolates produced with the alternative processing can be consumed not only as moulded chocolate but is also suitable for other applications, such as enrobing which requires smooth and thin chocolates (Afoakwa, 2010; Beckett, 2009).

<u>Thixotropy</u>

Another flow behaviour parameter that can be used to evaluate the performance of processing method is thixotropy. It was stated by Servais et al. (2004) and Afoakwa (2010) that well-conched chocolate should not exhibit thixotropic behaviour. Thus, the ability of a processing method to create chocolate with as low as possible thixotropy value is important. As can be seen in Table 4.6, similar to the chocolates formulated with cocoa mass, some chocolates formulated with cocoa powder and produced by alternative processing had thixotropy values higher than 1,5 Pa. Nevertheless, high thixotropy values were also observed in the chocolates produced by conventional processing. It seemed that the newly formed surface of sugar particles in the cocoa powder chocolate highly influenced the thixotropy values. Therefore, the extension of the liquefaction duration using the Stephan mixer can be tried to get more homogeneous chocolate suspension.

4.4.3 Microstructural properties

To be able to visualise the solid particles influencing the rheological behaviour of chocolate, molten chocolate and molten chocolate suspended in isopropanol were visualised under a microscope with polarised light and normal light, respectively. The results showed a clear variation in the microstructure of the chocolates. Normal light microscopic visualisation revealed that the particle size of the chocolate varied depending on the milling time (Figure 4.4). However, the impact of lecithin concentrations, ball sizes and cocoa sources on the solid particles could not be observed using this technique. The chocolates produced with 30 and 45 min milling time had smaller particles than the reference chocolates, whose particle sizes were comparable to those of the chocolates produced with 15 min milling time. Furthermore, it can be also seen in Figure 4.4 that particle agglomeration did not occur, supporting the fact that moisture was present in very low amount.

Figure 4.5 shows the appearance of the sugar crystals in the chocolates visualised under a polarised light microscope. It can be seen that the chocolate produced with 15 min milling time had bigger sized sucrose crystals (whitish appearance) than the chocolate produced with 30 and 45 min milling time. This observation was in accordance with the PSD data of the chocolates as mentioned in Table 4.5. In the same figure, it can be seen that the reference chocolates had also a comparable size of sucrose crystals to the chocolates produced with milling time of 15 min. Furthermore, in general, it can be observed in the Figure 4.5 that small particles filled the voids between the large particles, which may increase the maximum volume fraction to some extent. Moreover, irrespective of the cocoa source, it can be seen that the chocolate Ref 33% had slightly denser sucrose particles than Ref 36%. This observation was attributed to the fact that the chocolate Ref 33% had a lower fat content. Similar to the normal light microscopy, polarised light microscopy could not visualise the influence of lecithin concentrations, ball sizes and cocoa sources.

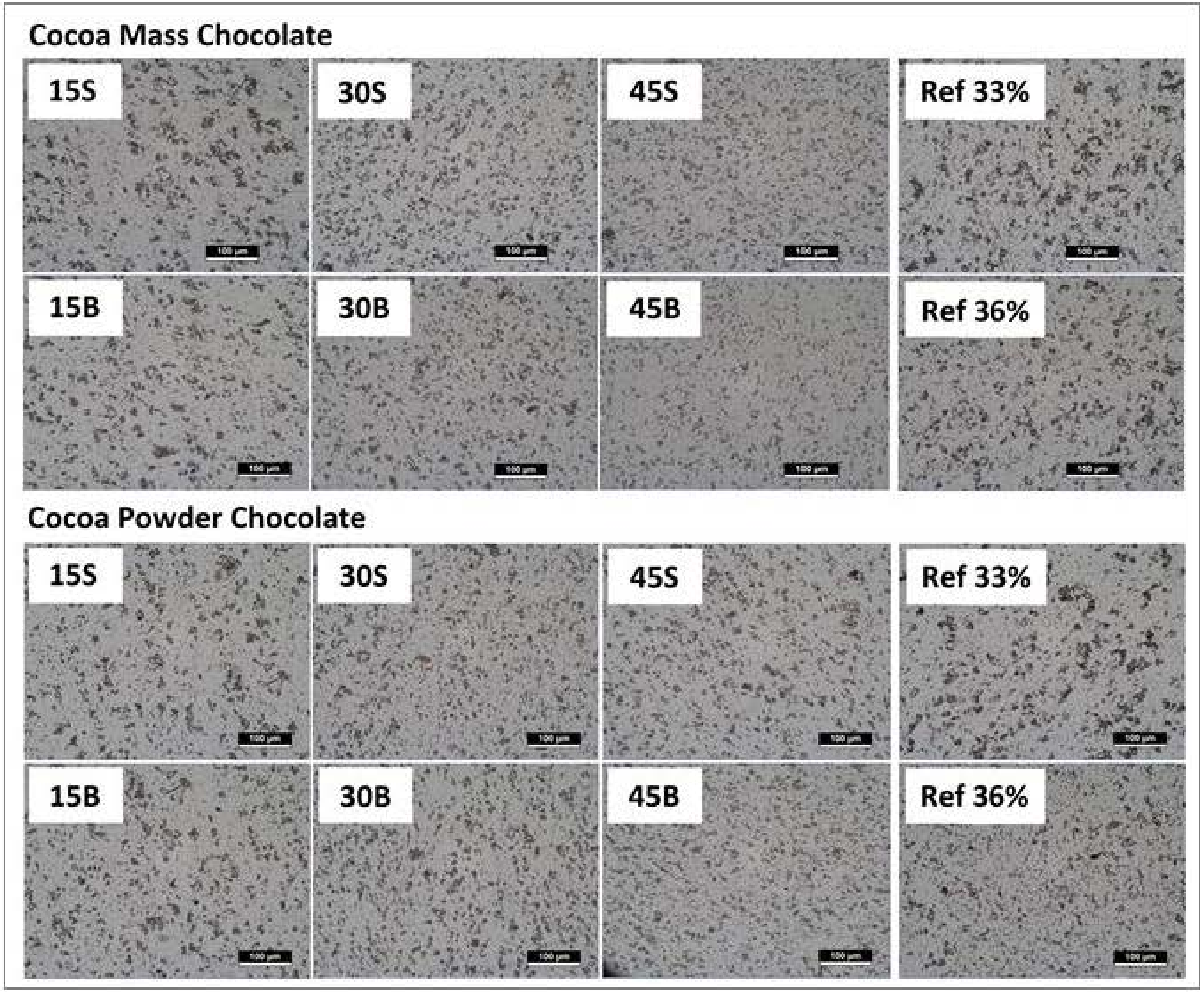

Figure 4.4 Solid particles of chocolate in isopropanol obtained with normal light microscopy, the scale bar represents 100 µm.

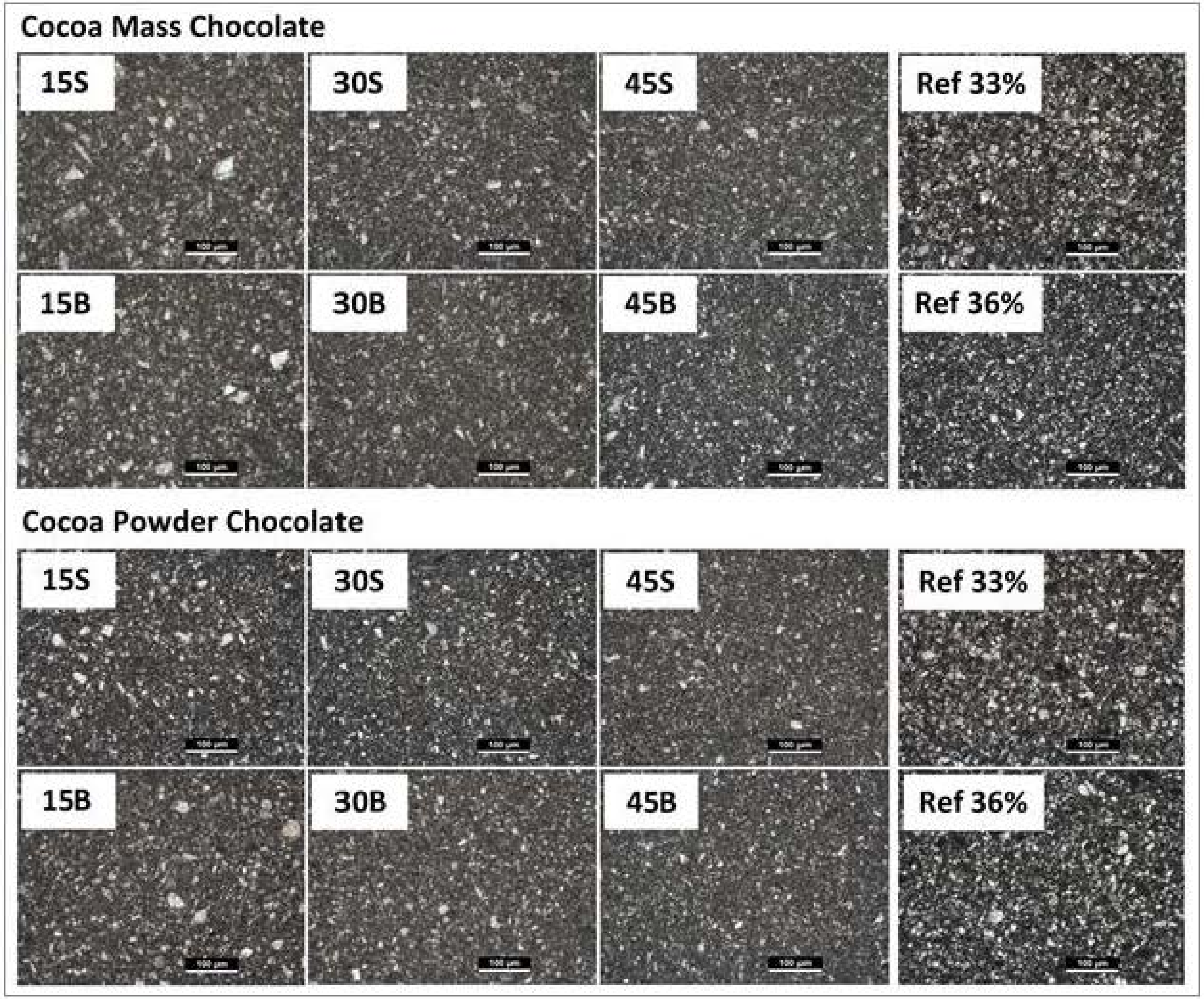

Figure 4.5. Micrographs of molten chocolates obtained with polarised light microscopy, the scale bar represents 100 µm.

4.5 CONCLUSIONS

Insights about experimental variables and their interactions that affected the PSD and the rheological behaviour of chocolates produced by alternative processing were obtained. It can be concluded that ball size and milling time together with their interaction were the most decisive experimental variables in reducing the size of solid particles. Further particle size reduction did not occur during liquefaction process. Regardless of the cocoa source, lecithin together with ball size and milling time as well as their interactions highly influenced the Casson yield value and Casson viscosity of the chocolates.

The results also showed that regardless of the cocoa source, the chocolates produced by alternative processing had comparable moisture content to the chocolates produced by conventional processing. Moreover, almost all cocoa butter trapped in the cocoa particles, irrespective of the cocoa source, was released. Even though ball mill processing does not have similar shearing system to the one in the conventional processing, chocolates produced using alternative processing had comparable free cocoa butter content to the chocolates produced using conventional processing.

Regardless of the cocoa source, the chocolates produced by alternative processing had higher Casson yield value and Casson viscosity than the chocolates produced by conventional processing, which might be attributed to a lower particle size and different surface morphology of the solid particles. Nevertheless, in general, those values were still in the range of acceptable chocolate viscosity. Microstructural visualisation could show the solid particle arrangement in the chocolate suspension, supporting the results of particle analysis by laser diffraction. From rheological and microstructural perspective, the alternative processing seemed to be suitable for small-scale chocolate industries. Irrespective of the ball size, the use of 30 min milling time and 0.4% lecithin were recommended. A longer liquefaction process can also be applied, to try to create more homogeneous chocolate mass.

CHAPTER 5

FEASIBILITY OF A SMALL-SCALE PRODUCTION SYSTEM APPROACH FOR PALM SUGAR SWEETENED DARK CHOCOLATE

This chapter is redrafted from :
Saputro AD, Van de Walle D, Kadivar S, Mensah MA, Van Durme J, Dewettinck K (2017). Feasibility of a small-scale production system approach for palm sugar sweetened dark chocolate. European Food Research and Technology, 243(6), 955-967.

CHAPTER 5
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5.1 ABSTRACT

Palm sugar is highly produced and patronised in the South Eastern part of Asia, *e.g.* Indonesia which is also the world's third largest cocoa producer. Recently, interest in palm sugar sweetened chocolate and better approaches for production methods that can be easily applied in developing countries are rising. This work investigated the influence of palm sugar on the quality attributes of dark chocolate in terms of fineness, rheological behaviour and aroma profile compared to that of sucrose. Furthermore, a small-scale processing approach using the combination of Stephan mixer and ball mill compared to the conventional method was investigated. The results showed that palm sugar sweetened chocolate exhibited a higher viscosity, a higher particle volume fraction and a higher degree of particle agglomeration due to its relatively high moisture and presence of glucose and fructose. Furthermore, chocolates sweetened with palm sugar displayed a distinctive aroma profile with the abundant presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) and pyrazine-based compounds. Applying the alternative processing method led to the production of chocolate with rather high degree of agglomeration and viscosity as compared to chocolate produced by means of conventional processing. Moreover, the alternative processing method resulted in the presence of myrcene, ß-trans-ocimene and isoamyl acetate which were not observed in the conventionally produced chocolates. The alternative processing method, however, seems to have potential for small scale production of dark chocolate sweetened with palm sugar.

5.2 INTRODUCTION

A sweetener that can be used as a natural alternative to sucrose is palm sugar which is highly produced and patronised in Southeast Asian countries. The utilisation of palm sugar will not only provide a new choice of alternative chocolate sweeteners, but also create distinct chocolate characteristics (Chapter 2; section 2.5). However, to ensure good quality of palm sugar sweetened chocolate, a suitable production method that can be easily applied should be developed.

Conventionally, molten chocolate is produced by a sequence of mixing, roll-refining and conching in relatively large scale machines. Mixing is the first and basic operation employed to obtain a constant formulation consistency. Refining is mainly aimed at reducing the particle sizes to below 30 µm which can be achieved using 2-, 3- or 5- roll refiner or the combinations. Conching is often a very time- and energy-consuming step in chocolate production, but in turn is crucial since the moisture reduction and fine-tuning of a desirable flavour profile occur in this step. Moreover, conching also helps covering the newly formed surfaces obtained from grinding with fat and, hence, improving the chocolate flow properties (Beckett, 2009).

Alternative processing methods which require less time and energy, and applicable for small scale chocolate production are highly required in Southeast Asian countries, *e.g.* Indonesia, Malaysia, Thailand, Philippines, *etc*. Thus, cocoa and/or palm sugar farmers with small chocolate industries will be able to produce chocolate on their own. Ball mill processing for instance is a method where both refining and mixing can be carried out in a single production system. Several studies related to ball mill processing with sucrose as sweetener have been conducted (Alamprese et al., 2007; Bolenz et al., 2006; Bolenz et al., 2014a; Bolenz and Manske, 2013; Bolenz et al., 2014b; Lucisano et al., 2006; Pajin et al., 2013). One of the drawbacks of using ball mill is the difficulty in moisture and acidity reduction. Therefore, to overcome this major drawback, Stephan mixing can be utilised in combination with ball mill processing. Aidoo (2015) reported that the Stephan mixer, equipped with vacuum pump, appeared promising to produce chocolate with desired rheological properties. In their finding, refined sucrose sweetened chocolate with fat content of 33%

exhibited Casson yield value of 9.5 Pa and Casson viscosity of 2.1 Pa.s, similar to the reference sample produced by conventional processing.

The aim of this study was to investigate the feasibility of an alternative method compared to the conventional process as chocolate production system. In addition, the influence of palm sugar on the fineness, rheological behaviour, and aroma profile of dark chocolate was studied and compared with the reference chocolate produced by sucrose. Therefore, the finding of this work will be very useful for the development of palm sugar sweetened dark chocolate manufacture, as a typical Southeast Asian chocolate.

5.3 MATERIALS AND METHODS

5.3.1 Raw materials

Cocoa liquor (CM1 IC B), cocoa butter (pure prime pressed cocoa butter CB 1) and soy lecithin (SLS-578-12) were obtained from Belcolade (Erembodegem, Belgium). Coarse sucrose (Kristalsuiker EU2) and fine sucrose were supplied by Tiense Suikerraffinaderij (Tienen, Belgium) and Barry Callebaut (Wieze, Belgium), respectively, while palm sugar was purchased from CV. Sari Nira Nusantara (Yogyakarta, Indonesia).

5.3.2 Sample preparation

Chocolates with 36% fat (wet matter basis) were prepared according to the following formulation: 48.0% sugar, 34.4% cocoa mass, 17.2% cocoa butter and 0.4% lecithin. Palm sugar, sucrose and cocoa mass used for the chocolate production contained 2.4%, 0.2% and 1.5% moisture, respectively. Thus, on dry matter basis, palm sugar sweetened chocolate contained 36.6% fat and chocolates sweetened with sucrose contained 36.3% fat.

Palm sugars (PS) with D(v,0.9) of 1170 µm and 300 µm were used as sweeteners for chocolates produced through alternative processing (AP) and conventional processing (CP), respectively. Similarly, coarse and fine sucrose (S) with D(v,0.9) of 925 µm and 280 µm were used as sweeteners in reference chocolates produced through AP and CP, respectively. The specifications of the sugars can be found in Table 5.1.

Table 5.1. Specification of sugars used for chocolate productions (dry matter basis)

Sugar	Glucose (%)	Fructose (%)	Sucrose (%)	Protein (%)	Density (g/cm^3)
PS	2.0	3.3	78.7	1.3	1.54
S	n.d.	n.d.	100	n.d.	1.57

- n.d : not detected
- Sugar composition, moisture content, protein level and density were obtained through gas chromatography, Karl Fischer titration, Kjeldahl method and a pycnometer, respectively

5.3.2.1 Alternative processing

Alternative processing consisted of three steps; mixing with Stephan Universal Machine UMC 5 (Stephan food service equipment GmbH, Hameln, Germany), refining using ball mill (Duyvis Wiener, Koogaan de Zaan, Netherlands) and liquefaction by Stephan mixer. The chocolate processing method was similar to the one described in Chapter 4 (Section 4.3.2.1) with refining time of 30 min.

The liquefaction step was conducted in Stephan mixer at temperature of 50°C and blade speed of 1500 rpm for 15 min. Prior to this step, 0.2% of extra lecithin was added into the refined chocolate. Afterwards, liquid chocolates were sampled in sealed plastic containers at ambient temperature and used for particle size distribution (PSD), rheological behaviour and moisture content analyses. All measurements were done in triplicate.

5.3.2.2 Conventional processing

Chocolates were produced conventionally on a 5 kg-scale through planetary Vema mixer BM 30/20 (NV Machinery Verhoest, Izegem, Belgium), Exakt 80S 3-roll refiner (Exakt Apparatebau, Norderstedt, Germany) and Buhler Elk'Olino conche (Richard Frisse GmbH, Bad Salzuflen, Germany). The chocolate processing method was similar to the one described by Tran et al. (2015b), except the dry conching temperature was at 70°C instead of 80°C (Chapter 4; Section 4.3.2.2). The same sampling procedure as outlined in Section 5.3.2.1 was applied.

5.3.2.3 Tempering and Moulding

Tempering was performed manually followed by measuring the temper index (TI) through a temper meter (Aasted Mikroverk Choco Meter MK-3, Farum, Denmark) to verify that the chocolates were tempered correctly ensuring contraction, desired gloss and snap. All the chocolates had TI in the range of 4 to 6 and a chocolate temper unit (CTU) of 22.5-24.6^{o}C, both indicative for well-tempered products. Afterwards, tempered chocolates were poured into moulds of 100 mm x 24 mm x 10 mm and subsequently cooled at 12°C for 1 hour. Afterwards, the chocolate bars were de-moulded and transferred to a thermal cabinet at 20^{o}C for 24 hours. Finally, the chocolate bars were wrapped with aluminium foil and allowed to mature at 20^{o}C. After 14 days of maturation, melting and aroma profile analyses were conducted in triplicate.

5.3.3 Analytical methods

5.3.3.1 Moisture content

Moisture content of sugars was measured according to the method described in Chapter 3 (Section 3.3.2.1). Moisture content of chocolates was determined following the method described in Chapter 4 (Section 4.3.3).

5.3.3.2 Particle size distribution (PSD)

PSD of chocolates was evaluated using a Malvern Mastersizer (Malvern Instruments Ltd, Worcestershire, UK) equipped with a 300 RF lens to measure particles in a range of 0.05 - 900 µm. Optional devices consisting of a vessel and a pump to circulate the isopropanol suspension were installed. Approximately 0.5 g of chocolate was diluted in 10 ml of isopropanol and heated in an oven at 55^{o}C for one hour. The diluted chocolate was then shaken vigorously prior to measurement. PSD measurements was carried out following two sample preparation protocols, namely without and with sonication. The latter was conducted by subjecting the diluted chocolate to ultrasound waves at 80 Hz for 15 min. D(v,0.9), D(v,0.5), D(v,0.1), D(4,3) and D(3,2), representing 90%, 50% and 10% percentiles, volume-weighed mean and Sauter diameter respectively were derived from the obtained data. Span, indicating distribution width, was also recorded.

5.3.3.3 Melting behaviour

Melting profiles of the sugars and chocolates were recorded with a Q1000 differential scanning calorimeter (DSC) equipped with a refrigerated cooling system (TA Instruments, New Castle, USA). The DSC was calibrated with indium, azobenzene and undecane, and aluminium cups were used. Approximately 5 mg of tempered chocolate after 14 days maturation was hermetically sealed in aluminium cups. The same amount of sample was used for the sugars. During thermal profiling, the samples were equilibrated at 20^{o}C followed by a heating step to 200^{o}C at a rate of 5^{o}C/min. Melting profile data integration was carried out with Universal Analysis 2000 software version 4.7A (TA Instruments) using linear baseline. Melting peaks were characterised by onset temperature (°C), maximum melting temperature (°C) and enthalpy (J/g). The ratio of enthalpy (%) was calculated using Equation 5.1, with 0.48 is the proportion of sugar in the chocolate formulation and m% is the percentage of sucrose and reducing sugar (glucose and fructose) present in the sweetener (Table 5.1).

$$Ratio\ of\ enthalpy\ (\%) \quad = \quad \frac{\dfrac{\Delta H\ sugar\ in\ chocolate\ \left(\frac{J}{g\ chocolate}\right)}{m\ \%\ x\ 0.48\ \left(\frac{g\ sugar}{g\ chocolate}\right)}}{\Delta H\ sugar\ \left(\frac{J}{g\ sugar}\right)} \qquad \text{(equation 1)}$$

5.3.3.4 Rheological behaviour

Rheological behaviour of chocolate was measured according to the method described in Chapter 4 (Section 4.3.3).

5.3.3.5 Aroma analysis

Aroma volatile profiles were analysed using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) as described by Tran et al. (2015a) and Van Durme et al. (2016). The isolation of volatiles was conducted in a multi-purpose sampler (Gerstel, Mulheim an der Rur, Germany) equipped with a HS-SPME unit. Two grams of chocolate was mixed with 3 µl internal standard undecane with a concentration of 0.368 µg/µl in a hermetically sealed 20 ml vial. The latter was then incubated for 10 min at 60^{o}C. Afterwards, a well-conditioned 50/30 µm Carboxen/Divinylbenzene/Polydimethylsiloxane (CAR/DVB/PDMS) SPME fibre (Supelco, Sigma-Aldrich N.V., Bornem, Belgium) was exposed to the headspace of the chocolates for 25 min at 60^{o}C. The volatiles were then analysed through GC-MS using splitless injection, helium as the carrier gas (1 ml/min) and ZB-WAX column of 30 m (length) x 0.25 mm (internal diameter) x 0.25 µm (film thickness) (Agilent Technologies, Diegem, Belgium). The following time-temperature program was applied:

holding at 35°C for 5 min, heated to 180°C at 5 °C/min followed by heating to 250°C at 10°C/min. Injector and transfer lines were maintained at 250°C and 280°C, respectively. The total ion current (70 eV) was recorded in the mass range from 40 to 250 amu (scan mode) using no solvent delay and a threshold of 50. Tentative identification of the volatile compounds in the headspace was performed using the Wiley 275 library.

5.3.4 Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). The chocolate properties were subjected to variance analysis (ANOVA) with 5% significance level. Testing for homogeneity of variances was performed with Levene Test. When the conditions for homogeneity of variances were fulfilled, Tukey test was used to determine differences among samples. In case variances were not homogeneous, Games–Howell test was performed. To visualise the relationships between chocolates and their volatile composition, principal component analysis (PCA) was performed.

5.4 RESULTS AND DISCUSSION

In this study, the feasibility of a small-scale production of dark chocolate sweetened with palm sugar through alternative processing (AP), was compared with conventional processing (CP). Moreover, the impact of palm sugar (PS) in comparison to sucrose (S) on the most important quality attributes of dark chocolate, namely fineness, rheological behaviour and aroma profile was investigated.

5.4.1 Impact of processing method and type of sweetener on the fineness of chocolate

In this research, PSD measurement was conducted following two different sample preparations, namely without and with sonication. Differences in PSD values following these treatments could then be attributed to the presence of particle agglomeration in the chocolate suspension. The higher the size reduction of the particles can be related to the higher degree of agglomeration.

It can be observed in Table 5.2 that, for the same chocolate processing method, chocolate sweetened with palm sugar exhibited a higher degree of agglomeration than the one sweetened with sucrose. Besides, for the same type of sweetener, chocolate produced by means of AP showed a higher degree of agglomeration than their counterparts produced through CP. Palm sugar sweetened chocolate obtained from alternative processing (PS AP) displayed the highest degree of agglomeration, followed by the one sweetened with palm sugar produced by conventional processing (PS CP). As reference, chocolate sweetened with sucrose produced through alternative processing (S AP) exhibited a relatively low degree of agglomeration, whereas, no agglomeration was observed in the sucrose sweetened chocolate obtained by conventional processing (S CP).

Palm sugar sweetened chocolate exhibited a higher degree of agglomeration than chocolates sweetened with sucrose. This results can be explained by the fact that palm sugar contains glucose and fructose (Apriyantono et al., 2002; Phaichamnan et al., 2010a) as hygroscopic materials, that might absorb moisture from the environment, resulting in a higher initial moisture content, contributing to agglomeration within the chocolate matrix. In addition, inadequate moisture evaporation during palm sugar drying, at the end of palm sugar production, might also contribute to a higher moisture content. The presence of moisture in chocolate either dissolves sugar particles or creates sticky patches on the surfaces of the sugar particles, inducing agglomeration (Beckett, 2008). Moreover, the fact that conventional processing removes moisture more effectively (Table 5.3), created chocolates with a lower particle agglomeration degree (Table 5.2).

Table 5.2. Comparison of particle size distribution parameters of non-sonicated and sonicated chocolates sweetened with palm sugar and sucrose

Chocolate	PSD (without sonication pre-treatment)					
	Derived Diameter (µm)		Distribution Percentiles (µm)			Span (-)
	D(3,2)	D(4,3)	D(v,0.9)	D(v,0.5)	D(v,0.1)	
PS AP	3.1 ± 0.1^{c}	22.6 ± 0.3^{c}	57.3 ± 0.6^{d}	13.0 ± 0.2^{c}	1.8 ± 0.0^{c}	4.3 ± 0.1^{b}
S AP	2.2 ± 0.2^{a}	8.1 ± 0.4^{a}	18.4 ± 1.0^{b}	5.5 ± 0.2^{a}	1.1 ± 0.0^{a}	2.8 ± 0.1^{a}
PS CP	2.4 ± 0.1^{ab}	7.8 ± 0.3^{a}	16.7 ± 0.5^{a}	5.6 ± 0.2^{a}	1.3 ± 0.0^{b}	2.9 ± 0.0^{a}
S CP	2.5 ± 0.1^{b}	9.1 ± 0.1^{b}	20.7 ± 0.1^{c}	6.6 ± 0.1^{b}	1.3 ± 0.0^{b}	2.8 ± 0.1^{a}

Chocolate	PSD (with sonication pre-treatment)					
	Derived Diameter (µm)		Distribution Percentiles (µm)			Span (-)
	D(3,2)	D(4,3)	D(v,0.9)	D(v,0.5)	D(v,0.1)	
PS AP	2.6 ± 0.3^{b}	11.2 ± 0.2^{c}	25.9 ± 1.1^{d}	7.9 ± 0.3^{d}	1.4 ± 0.2^{c}	3.1 ± 0.3^{b}
S AP	2.3 ± 0.1^{a}	7.3 ± 0.2^{a}	15.8 ± 0.3^{b}	5.4 ± 0.1^{b}	1.2 ± 0.1^{a}	2.7 ± 0.0^{a}
PS CP	2.3 ± 0.1^{a}	6.8 ± 0.2^{a}	14.5 ± 0.2^{a}	5.2 ± 0.1^{a}	1.2 ± 0.0^{a}	2.9 ± 0.0^{a}
S CP	2.6 ± 0.1^{b}	9.0 ± 0.3^{b}	20.4 ± 0.6^{c}	6.6 ± 0.2^{c}	1.3 ± 0.1^{b}	2.6 ± 0.1^{ab}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

5.4.2 Impact of processing method and type of sweetener on the melting behaviour of chocolate

To get better insights in the observed phenomenon mentioned in part 5.4.1, the thermal behaviour of the sugar phase in chocolate was recorded. It can be seen in Figure 5.1 that the DSC profile exhibited two endothermic peaks. The first peak, which is related to mouthfeel melting, can be attributed to the melting of cocoa butter, while the second peak beyond 140^{o}C can be associated to the melting of sugar. With regard to fat melting, all type of chocolates (PS AP, S AP, PS CP, S CP) displayed a similar melting peak temperature in the range of 33.7^{o}C to 34.0^{o}C, while in the case of sugar melting, regardless of the processing methods, palm sugar had a lower melting temperature than sucrose. According to Figure 5.1, chocolates formulated with palm sugar (PS AP and PS CP) exhibited significantly lower (p<0.05) onset and melting peak temperature than chocolates sweetened with sucrose (S AP and S CP). The melting peak temperature of PS AP and PS CP chocolates were 152.3°C and 156.8°C, respectively, whereas the melting peak temperature of S AP and S CP chocolates were 174.1°C and 177.4°C, respectively. Alternative processing caused less moisture evaporation than conventional processing (Table 5.3). The presence of more moisture, which acts as plasticizer (Beckett et al., 2006; Kedward et al., 1998b; Kedward et al., 2000), induced a lower sugar melting peak temperature in chocolate. Beckett et al. (2006) stated that the reduction in melting point of a substance can be correlated to the presence of impurities. Therefore, the presence of reducing sugars, proteins and minerals in palm sugar as "impurities", might contribute to a lower melting peak temperature of sugar phase in chocolate.

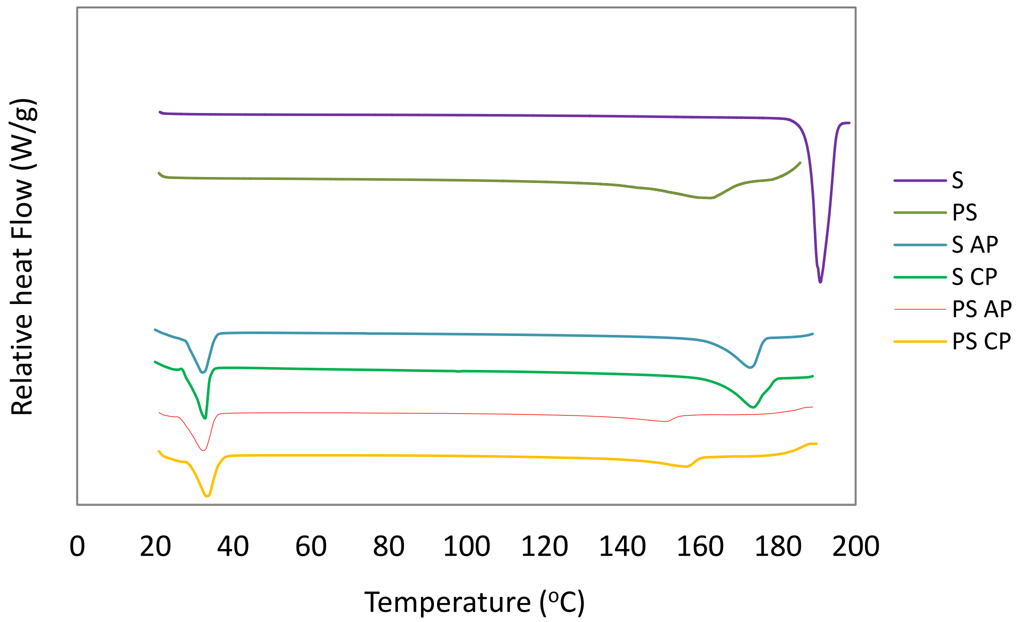

Figure 5.1. Melting profile of sugar (PS, S) and chocolates sweetened with palm sugar and sucrose produced by alternative processing (PS AP, and S AP) and conventional processing (PS CP, and S CP)

The presence of hygroscopic components, *e.g.* fructose and glucose, together with sucrose (Apriyantono et al., 2002; Ho et al., 2008b; Phaichamnan et al., 2010a) and relatively high moisture might trigger the development of additional amorphous sugar during refining. The formation of (extra) amorphous sugar will reduce the endothermic enthalpy of the sugar phase in chocolate. The amount of crystalline sugar in chocolates can be compared by determining the ratio of the melting enthalpy value of the sugar phase in chocolate ($\Delta H_{sugar\ in\ chocolate}$), corrected for sugar content, to the melting enthalpy value of raw ingredient (ΔH_{sugar}). The lower the ratio of enthalpy (%), as indicator of the presence of crystalline part, the higher the amount of amorphous sugar and/or moisture present in the chocolate. As can be seen in Figure 5.1, sucrose (S) exhibited a higher melting temperature than palm sugar (PS). A high degree of sucrose crystallinity is indicated by a sharp melting peak. Thus, a broader melting curve of palm sugar designated that palm sugar had a relatively low degree of crystallinity and contained chemical "impurities".

As hypothesised, palm sugar sweetened chocolate contained a higher amorphous fraction than sucrose sweetened chocolate. According to Table 5.4, chocolates sweetened with sucrose (S AP and S CP) exhibited significantly higher ratio of enthalpy ($p < 0.05$) than chocolates sweetened with palm sugar (PS AP and PS CP). In addition, irrespective of the sweetener type, alternative processing resulted in chocolate with a lower ratio of enthalpy (%) than conventional processing.

Generally, the presence of amorphous sugar in chocolate could also be observed through a glass transition, highly influenced by moisture acting as plasticiser (Ergun et al., 2010). However, as can be derived from Figure 5.1, the glass transition of sugar in chocolate could not be observed. This phenomenon might be due to an overlap between the glass transition and cocoa butter melting or instrument sensitivity. DSC might not be sensitive enough to detect glass transitions of sugar particles dispersed in a fat phase.

5.4.3 Impact of processing method and type of sweetener on the rheological behaviour of chocolate

The rheological behaviour of molten chocolate is crucial in chocolate applications such as enrobing, shell formation and moulding (Servais et al., 2004). Rheologically, molten chocolate exhibits a non-ideal plastic behaviour, more particularly shear-thinning occurs once a yield value has been overcome (Afoakwa, 2010). One of the models that can be used to fit this non-ideal plastic behaviour is the Casson model, from which the Casson yield stress (σ_{CA}) and Casson viscosity (η_{CA}) are derived. At the same fat and lecithin content, the flow behaviour of chocolate is mainly influenced by particle size distribution, particle volume fraction, and moisture content (Afoakwa, 2010; Beckett, 2009; Do et al., 2007; Mongia and Ziegler, 2000). Degree of agglomeration as the result of the presence of moisture and amorphous sugar also impacts the chocolate flow parameters to some extent.

Table 5.3. Moisture content of ingredients, moisture content of chocolates, evaporated moisture during processing and final fat content of chocolates after moisture evaporation during processing.

Chocolate	Moisture Content (%)			Final fat content of chocolate after moisture evaporation during processing (% wmb)
	Total Moisture of ingredients (%)	Moisture content of chocolate (%)	Evaporated moisture (%)	
PS AP	1.67 ± 0.04[b]	1.51 ± 0.00[d]	0.15 ± 0.04[b]	36.05 ± 0.02[a]
S AP	0.64 ± 0.01[a]	0.65 ± 0.02[b]	0.01 ± 0.02[a]	36.00 ± 0.01[a]
PS CP	1.67 ± 0.04[b]	0.81 ± 0.03[c]	0.86 ± 0.03[c]	36.31 ± 0.01[b]
S CP	0.64 ± 0.01[a]	0.44 ± 0.03[a]	0.21 ± 0.03[b]	36.07 ± 0.01[a]

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples
wmb: wet matter basis

Table 5.4. Ratio of enthalpy (%) of chocolates sweetened with palm sugar and sucrose produced by alternative processing (PS AP, and S AP) and conventional processing (PS CP, and S CP)

Chocolate	Enthalpy (J/g)		Ratio of enthalpy (%)
	Sugar	Chocolate	
PS AP	97.5 ± 3.1[a]	14.6 ± 0.5[a]	39.1 ± 1.8[a]
S AP	163.8 ± 1.7[b]	47.1 ± 1.4[c]	60.1 ± 2.2[c]
PS CP	97.5 ± 3.1[a]	21.8 ± 2.2[b]	55.3 ± 3.9[b]
S CP	159.6 ± 4.5[b]	49.8 ± 0.8[c]	65.2 ± 2.4[c]

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

The rheological behaviour in the studied samples was dependent on the processing method and type of sweetener. Alternative processing which was less effective in removing moisture than conventional processing (Table 5.3), led to a higher degree of agglomeration. This seemed to affect the Casson yield value, Casson viscosity, and thixotropy of molten chocolate. In addition, the utilisation of palm sugar also influences the rheological behaviour of chocolates. The fact that the initial moisture content of palm sugar was higher than sucrose, contributed to particle agglomeration in the chocolates. Aside from this, due to the low density of palm sugar, it created chocolate with a higher particle volume fraction which resulted in more particle-particle interaction, leading to an increase in the Casson viscosity of molten chocolate.

It can be observed in Table 5.5 that PS CP and S AP chocolates exhibited significantly higher yield value ($p < 0.05$) than PS AP and S CP chocolate. Nevertheless, it can also be observed that S AP showed significantly higher ($p <0.05$) yield value than PS CP. This observation can be mainly attributed to the combination of particle size distribution (PSD), degree of agglomeration, and maximum volume fraction (packing fraction). With regard to the PSD data (Table 5.2), S AP and PS CP displayed significantly ($p < 0.05$) lower D(v,0.9) and D(3,2) value than PS AP and S CP. D(v,0.9) was directly proportional to D(v,0.5), D(v,0.1) and Sauter mean D(3,2), but inversely related to specific surface area of particles. This implies that S AP and PS CP had smaller particles and higher surface area resulting in stronger particle-particle interaction in the chocolate suspension. Mongia and Ziegler (2000) stated that at low shear stresses, close to the yield value, the rheological behaviour of chocolate is mainly dominated by particle-particle interactions. The smaller the particle size, the larger the specific surface area, thus, the stronger the particle-particle interactions are formed, resulting in a higher yield value. Aside from this, increased final fat content due to moisture evaporation during processing may also have affected the chocolate flow behaviour. The fact that PS CP had a lower yield value than S AP can be attributed to the increased fat content of PS CP after processing. It can be seen in Table 5.3 that PS CP, which exhibited the highest moisture evaporation during processing, contained slightly more fat than the other chocolates. Thus, even though PS CP had a slightly lower D(v,0.9) than S AP, the yield value of PS CP was lower than S AP. A higher amount of fat reduces particle-particle interaction in the chocolate suspension.

Table 5.5. Rheological behaviour parameters of chocolates sweetened with palm sugar and sucrose produced by alternative processing (PS AP, and S AP) and conventional processing (PS CP, and S CP)

Chocolate	Rheological behaviour parameters		
	Casson yield value (Pa)	Casson viscosity (Pa.s)	Thixotropy (Pa)
PS AP	3.4 ± 0.2[a]	3.3 ± 0.1[c]	2.7 ± 0.1[c]
S AP	15.9 ± 0.2[c]	2.2 ± 0.1[b]	1.3 ± 0.1[b]
PS CP	12.4 ± 0.1[b]	2.3 ± 0.1[b]	3.0 ± 0.3[c]
S CP	3.5 ± 0.0[a]	1.3 ± 0.1[a]	0.5 ± 0.1[a]

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

Furthermore, it can be observed in Table 5.2 that PS AP exhibited the highest value of span (distribution width), resulting in the lowest yield value (Table 5.5). Theoretically, a wider PSD span leads to a higher maximum particle volume fraction (Do et al., 2007; Servais et al., 2002). This may result in a lower yield value. Apart from this, the degree of agglomeration, induced by the presence of moisture and reducing sugars, also influenced the yield value to some extent. Thus, in this case, even though the particle density of palm sugar was lower than that of sucrose, the influence of agglomerates surface area was more pronounced than the influence of particle density. Therefore, the yield value of PS AP was lower than that of the other chocolates.

Casson viscosity of the chocolates were largely influenced by combination of particle density and moisture content. In this case, the influence of agglomerates surface area to the Casson viscosity was less pronounced than their influence on the yield value. S CP which had the lowest moisture content (Table 5.3) and the highest particle density (Table 5.1) showed to have the lowest Casson viscosity. Conversely, PS AP with the highest moisture content (Table 5.3) and the lowest particle density (Table 5.1), exhibited the highest Casson viscosity. Chocolates with a high moisture content require more fat to coat the solid particles, which reduce the availability of “free” fat for the movement, resulting in high viscosity. Beckett (2008) stated that for every 0.3% extra moisture left in chocolate at the end of conching, an extra of 1% fat should be added to attain similar flow properties. For this reason, a slightly increased fat content of PS CP (Table 5.3) seemed insufficient to provide additional “free” fat for the movement. In addition, the differences in chemical “impurities” (reducing sugar, protein, minerals) among palm sugars, might affect the particle interaction which will further influence the chocolate viscosity. Additionally, conventional processing might release more fat from cocoa solids, resulting in somewhat higher free fat content of chocolate and reducing the viscosity of chocolate.

Another flow parameter illustrating chocolate quality is thixotropy. Do et al. (2007) stated that thixotropy is an indicator of the degree of particle agglomeration in a suspension. Servais et al. (2004) stated that a well-conched chocolates should not exhibit thixotropic behaviour. It can be observed from Table 5.5 that sucrose sweetened chocolates (S AP and S CP) exhibited significantly lower ($p<0.05$) thixotropy than chocolates sweetened with palm sugars (PS AP and PS CP). The presence of high moisture content which induces agglomeration is the most plausible reason for this phenomenon.

5.4.4 Impact of processing method and type of sweetener on the aroma profile of chocolate

The utilisation of palm sugar as sweetener in chocolate has an impact not only on the sweetness but also on the aroma profile. Table 5.6 presents the key volatiles in chocolates sweetened with palm sugar. Regardless of the processing methods, there were some volatiles which were present in chocolates sweetened with palm sugar (PS AP and PS CP), but were absent in the chocolates sweetened with sucrose (S AP and S CP). These include 2-furoic acid (odourless), DHM (2,3-dihydro-5-dihydroxy-6-methyl-4H-pyran-4-one), and DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one), the latter 2 volatiles conferring a caramel/roasted aroma (Bonvehi, 2005; Krings et al., 2006; Preininger et al., 2009). DDMP is an anti-oxidative and anti-carcinogenic compound which is easily formed in low moisture products through Maillard reaction (Ban et al., 2007; Seo et al., 2006b; Yu et al., 2013b).

The processing method was also found to influence the aroma volatiles detected in the chocolates. There were some key odourants which were only present in chocolates produced by alternative processing, but were not detected in the chocolates produced by conventional processing. Those volatiles were myrcene, ß-trans-ocimene, and isoamyl acetate which confer spicy, herbal/sweet and fruity notes, respectively (Kadow et al., 2013; Ramos et al., 2014), and also 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine which confer a sweet, caramel and roasted aroma (Counet et al., 2002a; Owusu et al., 2012; Rodriguez-Campos et al., 2012). Moreover, in general, PS AP chocolates exhibited significantly higher ($p < 0.05$) concentration of aroma volatiles than the respective chocolate sweetened with sucrose (S AP) (Table 5.6). Furthermore, regardless of odour threshold, the concentration of the volatiles in PS AP was significantly higher ($p < 0.05$) than the volatiles in PS CP and S CP. Sour, sweet and caramel/roasted aromas in the PS AP chocolates were present in high concentration as a result of the high concentration of acetic acid, 2(3H)-dihydrofuranone, 2,3-butanediol, and some alkylpyrazines. In contrast, the nutty, flowery, fruity and floral aroma, due to the presence of limonene, 2-nonanone, 2-heptanone, octanal, phenylethyl alcohol, 2-pentylfuran, benzaldehyde, 2-pyrrolidinone, and 2-acetylpyrrole were present in a lower concentrations.

The presence and/or absence of volatiles and different concentration of volatiles in the studied chocolates (Table 5.6) can be attributed to the different composition of sugars and/or different processing applied. Aroma volatiles which are present in palm sugar are likely to appear in palm sugar chocolates, contributing to the amount of certain aroma volatile compounds in chocolates. According to Ho et al. (2006; 2007), palm sugar contains DDMP, pyrazine-based compounds, acetic acid, aldehydes and furan. These volatiles might be derived from its raw material and also from Maillard reactions during sugar production (Apriyantono et al., 1996; Purnomo, 2007). In addition, as palm sugar contains not only sucrose but also fructose, glucose, protein and relatively high amounts of moisture (Apriyantono et al., 2002; BPS, 2010; BSN, 1995), this might induce additional chemical reactions during conching resulting in the presence of different volatiles in PS AP and PS CP chocolates compared to sucrose sweetened chocolates (S AP and S CP).

Effects of conching on the aroma of chocolates have been reported (Afoakwa et al., 2008c; Counet et al., 2002a; Owusu et al., 2012). Hoskin and Dimick (1983) reported that during conching, the temperature applied and/or the concentrations of free amino acids and sugars would be too low for Maillard reactions. However, Pontillon (1995) suggested that caramelization of lactose as reducing sugar and Maillard reactions with milk proteins might occur in milk chocolate. The latter shows that the addition of protein and also reducing sugars in chocolate mixture might induce additional Maillard reaction during chocolate production. In this study, the role of fructose and glucose as reducing sugars might be the same as the role of lactose in milk chocolate, while, the presence of protein in palm sugar, even in low concentration, might simulate the role of milk protein in milk chocolate. The result (Table 5.6) showed that PS AP chocolate exhibited significantly higher ($p < 0.05$) concentration of aroma volatiles than that of chocolate produced conventionally (PS CP). This phenomenon can be attributed to the fact that the conching time of conventional processing is approximately 4 hours longer than that of the alternative processing method, resulting in more aroma volatiles and moisture evaporation. It seemed that additional Maillard reactions was at least not substantial. The presence of reducing sugars and proteins in palm sugar, as precursors, also temperature and moisture content seemed not enough to significantly induce Maillard reaction during conventional chocolate production, and more particularly conching at 70°C for 6.5 h. In addition, the fact that alternative processing evaporated less moisture than conventional processing led to the formation of a more amorphous sugar during chocolate production, resulting in an increased level of palm sugar and cocoa aroma absorption. Beckett (2008) stated that if sugar is milled together with cocoa, some of the volatile cocoa flavours are absorbed by the amorphous sugar, resulting in a more intense flavour chocolate.

PCA data analysis is shown in Figure 5.2. It can be observed that PCA explained more than 89% variance in the first 2 factors, with PC1 73.6% and PC2 16.2%. In general, the distinction between alternative processing and conventional processing can be observed along PC1, while the difference between palm sugar and sucrose utilisation can be identified through PC2. Chocolates produced by means of alternative processing, in contrast to the ones produced by conventional processing, could be characterised by high positive value in PC1 which exhibited high aroma volatile concentrations of pyrazine-based compounds, 2-nonanone, 2-pentylfuran, myrcene, and ß-trans-ocimene, while chocolate formulated with palm sugar, counter to the ones formulated with sucrose, could be characterised by high positive values in PC2 showing high aroma volatile concentrations of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one(DDMP), 2,3-dihydro-5-dihydroxy-6-methyl-4H-pyran-4-one (DHM), octanal, pentanal, heptanal, and 2-acetylpyrrole.

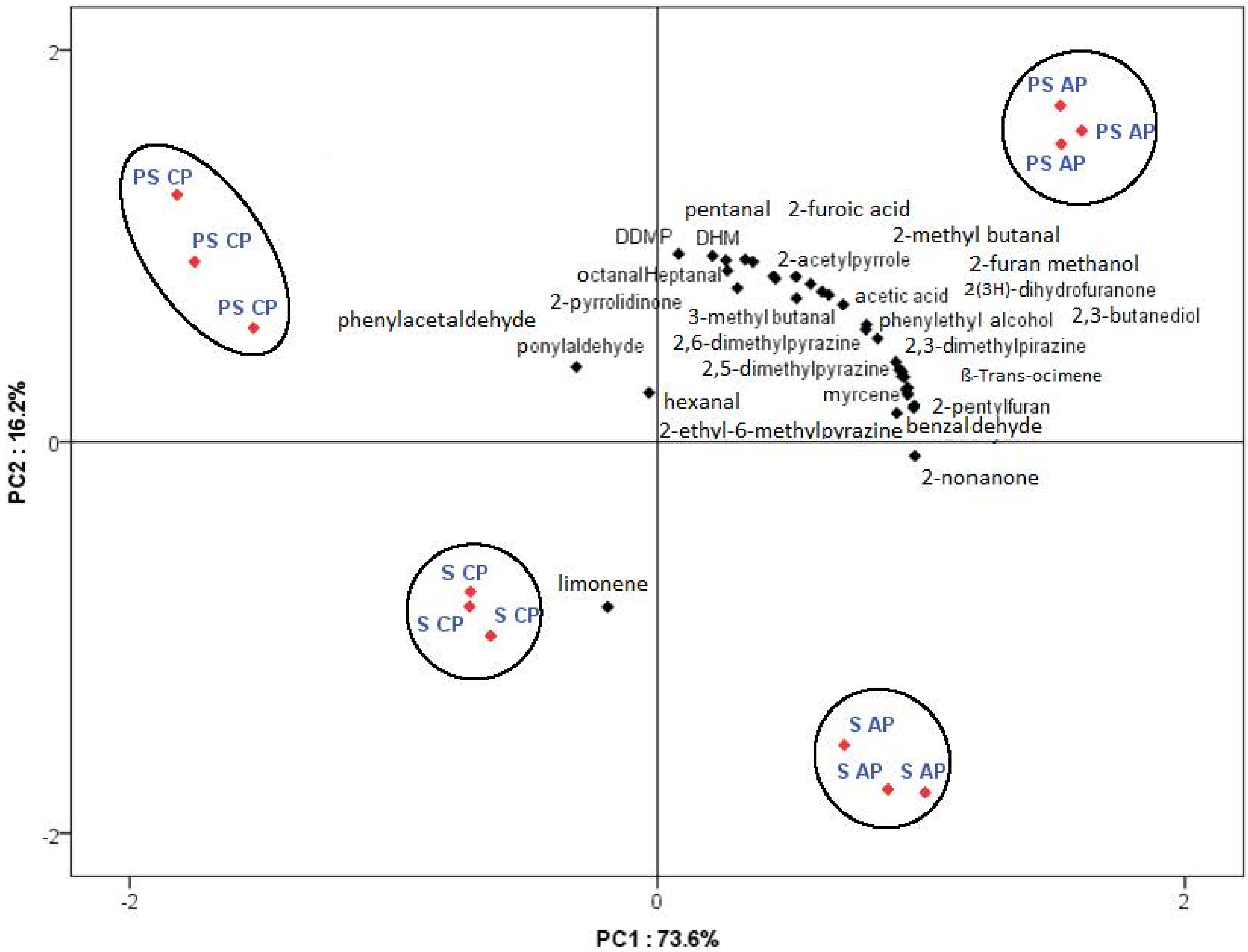

Figure 5.2. PCA (loadings and score plot) of aroma volatiles in chocolates sweetened with palm sugar and/or sucrose produced by means of alternative and/or conventional processing.

Table 5.6. Mean semi-quantitative concentrations of aroma volatiles (ng/g) in chocolates sweetened with palm sugars and sucrose identified by HS-SPME-GC-MS

Aroma Volatiles	Odour Description	References	Concentration (ng/g)			
			PS AP	S AP	PS CP	S CP
a. Pyrazines						
2,3-Dimethylpirazine	hazelnut, roasted, caramel, sweet	(Owusu et al., 2012), (Counet et al., 2002a), (Rodriguez-Campos et al., 2012)	29.6 ± 1.9[b]	17.3 ± 3.0[a]	n.d	n.d
2,5-Dimethylpyrazine	popcorn, roasted, cooked	(Owusu et al., 2012), (Afoakwa et al., 2009a)	81.0 ± 0.2[d]	30.7 ± 1.0[c]	19.8 ± 0.0[b]	10.9 ± 3.2[a]
2,6-Dimethylpyrazine	roasted, cooked	(Afoakwa et al., 2009a)	62.8 ± 2.7[c]	12.2 ± 0.3[b]	11.6 ± 1.1[b]	5.5 ± 1.2[a]
Ethylpyrazine	hazelnut, nutty, roasted	(Afoakwa et al., 2008c), (Afoakwa et al., 2009a)	18.6 ± 0.1[d]	11.9 ± 0.8[c]	3.9 ± 0.7[a]	6.2 ± 1.4[b]
2,3,5-Trimethylpyrazine	roasted nuts, peanut	(Bonvehi, 2005)	101.0 ± 1.4[c]	63.9 ± 5.0[b]	27.6 ± 2.4[a]	34.1 ± 4.8[a]
2,3,5,6-Tetramethylpyrazine	milk coffee, mocha, roasted, green	(Afoakwa et al., 2008c), (Counet et al., 2002a), (Rodriguez-Campos et al., 2012)	435.3 ± 24.2[d]	312.0 ± 24.7[c]	86.7 ± 5.8[a]	135.7 ± 5.0[b]
2-Ethyl-6-methylpyrazine	earthy, roasted	(Owusu et al., 2012)	13.2 ± 0.9[b]	6.4 ± 0.3[a]	n.d	n.d
2-Pyrrolidinone	Popcorn	(Krings et al., 2006)	43.5 ± 7.5[b]	6.1 ± 1.1[a]	17.2 ± 1.7 [c]	14.2 ± 2.4[b]
b. Aldehydes						
2-Methyl butanal	chocolate	(Afoakwa et al., 2008c), (Afoakwa et al., 2009a)	29.3 ± 0.8[c]	7.8 ± 2.0[a]	14.5 ± 1.9[b]	12.5 ± 0.8[b]
3-Methyl butanal	chocolate	(Afoakwa et al., 2008c), (Afoakwa et al., 2009a)	75.0 ± 2.4[c]	26.4 ± 4.8[a]	34.6 ± 2.2[b]	41.3 ± 0.7[b]
Pentanal	pungent	(Aprotosoaie et al., 2016)	45.6 ± 1.2[d]	16.8 ± 1.5[a]	31.9 ± 2.6[c]	23.2 ± 2.6[b]
Hexanal			68.0 ± 2.7[a]	60.7 ± 2.5[a]	63.8 ± 17.1[a]	69.0 ± 2.1[a]
Heptanal	Green, fermented	(Owusu et al., 2012)	20.5 ± 2.0[c]	11.1 ± 0.7[a]	15.7 ± 4.0[b]	12.1 ± 1.2[a]
Octanal	fruity, green	(Owusu et al., 2012), (Zellner et al., 2008)	67.1 ± 1.4[c]	23.5 ± 0.5[a]	50.7 ± 10.2[b]	22.5 ± 2.9[a]
Nonanal			58.5 ± 2.6[a]	49.8 ± 5.6[a]	60.5 ± 11.7[a]	60.1 ± 14.0[a]
Benzaldehyde	earthy, nutty	(Owusu et al., 2012), (Afoakwa et al., 2009a)	172.3 ± 9.7[c]	106.7 ± 28.5[b]	19.0 ± 2.3[a]	94.2 ± 14.1[b]
Phenylacetaldehyde	Flowery, sweet, honey	(Afoakwa et al., 2009a), (Owusu et al., 2012), (Counet et al., 2002a)	22.1 ± 5.0[c]	8.7 ± 1.2[ab]	16.1 ± 5.8[bc]	n.d

c. Furans						
2-Pentylfuran	musty, green	(Owusu et al., 2012)	32.5 ± 1.4[d]	24.9 ± 1.3[c]	11.5 ± 2.2[a]	16.0 ± 0.2[b]
2(3H)-Dihydrofuranone	Sweet	(Owusu et al., 2012)	131.9 ± 4.2[c]	41.7 ± 2.6[a]	52.2 ± 2.2[b]	40.8 ± 4.6[a]
2-Furan methanol	caramel, sweet, faint burning	(Afoakwa et al., 2009a), (Ramos et al., 2014)	197.6 ± 2.3[c]	36.4 ± 0.7[a]	65.7 ± 4.8[b]	32.3 ± 4.8[a]
2-Furoic acid	odourless	(Bonvehi, 2005)	95.1 ± 1.0[b]	n.d	43.3 ± 2.9[a]	n.d
d. Alcohols						
2,3-Butanediol	sweet chocolate	(Ramos et al., 2014)	1292.0 ± 48.1[d]	285.9 ± 4.4[a]	388.5 ± 19.6[b]	497.0 ± 35.5[c]
Phenylethyl alcohol	honey, spice, rose, flowery, caramel	(Rodriguez-Campos et al., 2011)	190.0 ± 8.9[c]	106.6 ± 5.7[b]	78.2 ± 3.6[a]	86.9 ± 3.9[a]
e. Terpenes						
Myrcene	spicy	(Kadow et al., 2013)	16.5 ± 0.3[b]	10.2 ± 0.3[a]	n.d	n.d
Limonene	fruity, citrus	(Piggoit and Paterson, 1994)	19.2 ± 0.4[a]	44.8 ± 2.5[c]	28.6 ± 1.8[b]	60.0 ± 1.3[d]
ß-Trans-ocimene	herbal, sweet	(Kadow et al., 2013)	24.4 ± 0.5[b]	14.5 ± 1.5[a]	n.d	n.d
f. Acids, Esters, Ketones, Pyrans, Pyrroles						
Acetic acid	sour, vinegar, astringent	(Owusu et al., 2012), (Afoakwa et al., 2009a), (Rodriguez-Campos et al., 2012)	10239.6 ± 134.1[d]	2823.0 ± 129.3[b]	3435.2 ± 161.9[c]	2359.3 ± 204.9[a]
2-Heptanone	fruity, floral green	(Owusu et al., 2012), (Rodriguez-Campos et al., 2012)	16.3 ± 0.5[d]	10.2 ± 0.6[c]	3.9 ± 0.8[a]	7.6 ± 0.7[b]
2-Nonanone	floral, fruity, green	(Zellner et al., 2008), (Kadow et al., 2013)	35.6 ± 0.9 [a]	33.9 ± 2.4 [bc]	4.1 ± 0.6 [d]	10.9 ± 1.4 [c]
2-Acetylpyrrole	hazelnut, popcorn, musty, nutty	(Rodriguez-Campos et al., 2012), (Tran et al., 2015a)	72.2 ± 2.0[c]	21.9 ± 1.4[a]	34.7 ± 1.0[b]	20.9 ± 1.6[a]
Isoamyl acetate	fruity, banana	(Ramos et al., 2014)	38.8 ± 2.6[b]	18.2 ± 1.2[a]	n.d	n.d
2,3-Dihydro-5-Dihydroxy-6-Methyl-4H-pyran- 4- one (DHM)	caramel, sweet	(Preininger et al., 2009)	78.6 ± 3.0[b]	n.d	38.5 ± 0.4[a]	n.d
2,3-Dihydro-3,5-dihydroxy-6-Methyl-4 H-pyran-4- one (DDMP)	roasted, caramel	(Bonvehi, 2005), (Krings et al., 2006)	1798.3 ± 177.2[b]	n.d	1422.7 ± 115.6[a]	n.d

Mean values ± standard deviations
Different superscripts per component indicate significant differences ($p < 0.05$) among samples
n.d : not detected

5.5 CONCLUSION

Palm sugar sweetened dark chocolate as compared to sucrose sweetened dark chocolate exhibited higher agglomeration which affects the fineness of the chocolate, and higher viscosity due to a relatively higher moisture level and lower particle density. In terms of processing methods, alternative processing seems to be less effective in removing moisture than the conventional one.

Regardless of the processing method, 2,3-dihydro-3,5-dihydroxy -6-methyl-4(H)-pyran-4-one (DDMP), an anti-oxidative and anti-carcinogenic compound, was only observed in chocolate sweetened with palm sugar and was not detected in the chocolate sweetened with sucrose. In terms of processing methods, palm sugar sweetened chocolate produced by means of alternative processing contained a relatively higher concentration of acetic acid, 2(3H)-dihydrofuranone, 2,3-butanediol, and some alkylpyrazines which confer sour, sweet, and caramel/roasted aroma. In addition, myrcene, ß-trans-ocimene, isoamyl acetate which confer spicy, herbal/sweet, and fruity aroma were observed only in the chocolates produced by means of alternative processing. The presence of more amorphous sugar in chocolate produced by alternative processing might explain this phenomenon. Aside from this, less intense evaporation in the alternative processing might also contribute to the presence of more aroma volatiles in the chocolates.

Findings from this work will provide a more cost effective means of chocolate production with shorter processing times for farmers and small chocolate industries in cocoa and palm sugar producing countries. Future studies need to focus on further improvements in the alternative processing method to remove more moisture and acid volatiles resulting in high quality palm sugar sweetened dark chocolate with distinct aroma profiles.

CHAPTER 6

QUALITY ATTRIBUTES OF DARK CHOCOLATES FORMULATED WITH PALM SAP SUGAR AS NATURAL ALTERNATIVE SWEETENER

This chapter is redrafted from :
Saputro AD, Van de Walle D, Aidoo RP, Mensah MA, Delbaere C, De Clercq N, Van Durme J, Dewettinck K (2016). Quality attributes of dark chocolates formulated with palm sap-based sugar as nutritious and natural alternative sweetener. European Food Research and Technology, 243(2), 177-191.

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6.1 ABSTRACT

Consumer demand for healthier alternative sweeteners and attempts to replace sucrose, the most common sweetener used in chocolate, is increasing in recent years. One sucrose alternative that has not been fully explored in chocolate is palm sap sugar. This work investigated the impact of sucrose replacement by coconut sugar (CCS1 and CCS2) and palm sugar (CPS1, CPS2 and CPS3) on the quality attributes of dark chocolate, more particularly colour, hardness, flow behaviour and aroma profile. The results showed that chocolates formulated with palm sap sugar were lighter in colour and harder than the reference chocolate made with sucrose, which could be attributed to a lower particle density and a higher moisture of palm sap sugar than those of sucrose. Analysis of the major volatile compounds recorded the presence of 2,3‑dihydro‑3,5‑dihydroxy‑6‑methyl‑4(H)‑pyran‑4‑one (DDMP) and high concentration of pyrazine-based compounds in the palm sap sugar sweetened chocolates. The former compound (DDMP) was, however, absent in the sucrose sweetened dark chocolate. The physicochemical properties of the sugars also had a significant effect on the rheological behaviour of the final chocolates with chocolates formulated with coconut sugar recording the highest Casson viscosity. With regard to fat melting, chocolates sweetened with palm sap sugar and sucrose exhibited similar melting range temperature. Palm sap sugar nevertheless seems to have great potential for dark chocolate applications with additional health benefits.

6.2 INTRODUCTION

Dark chocolate, according to the European legislation (2000/36/EC, 23 June 2000), is a confectionery product containing not less than 35% of total dry cocoa solids, 18% of cocoa butter and 14% of dry non-fat cocoa solids with addition of any sugar. This allows chocolate producers to replace sucrose, the most common sweetener used in chocolate, with other sweeteners. Several researches about sucrose replacement in chocolate have been conducted. Some of these are related to the usage of sugar alcohols, low-calorie polysaccharides and/or intense sweeteners (Aidoo et al., 2014 ; Belscak-Cvitanovic et al., 2015; Shah et al., 2010; Sokmen and Gunes, 2006)

Nowadays, the demand for healthier sweeteners in chocolate (and foods in general), is increasing. Health issues related to high sugar levels and calories are a major concern (Aidoo et al., 2014 ; Anton et al., 2010; Prakash et al., 2008). This situation compels food technologists to seek healthier alternatives for common mono- and/or disaccharides. Palm sap sugar is claimed to be a healthier alternative sweetener to sucrose because it contains minerals and vitamins (Philippine Coconut Authority, 2016), antioxidant (Naknean and Meenune, 2011b) and also exhibits low glycemic index (GI: 35-42) (Srikaeo and Thongta, 2015; Trinidad et al., 2010) compared to pure sucrose (GI: 58-82) (Foster-Powell and Miller, 1995). In addition, palm sap sugar also exhibits lower GI than that of a commercial milk chocolate (GI: 49) (Miller et al., 1995). Hence, this type of sugar might be better consumed by people with high blood sugar levels. Moreover, Ho et al (Ho et al., 2006b) reported that palm sap sugars contain 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP). DDMP, which has a caramel-like flavour, is a Maillard reaction product which exhibits antioxidant activity (Cechovska et al., 2011; Lerma et al., 2010; Yu et al., 2013a, b; Zhou et al., 2014) and also has potency to reduce the risk for colon cancer (Ban et al., 2007).

The presence of reducing sugars and amino acids in palm sap sugar might induce additional Maillard reactions during chocolate production, contributing to the development of a distinct chocolate aroma. Nevertheless, undesirable Maillard reaction products such as acrylamide will not be formed or will be formed in very low levels during palm sugar and chocolate production. Thus the use of palm sap sugar in chocolate production will not give negative effect. Palm sap sugar is produced at temperatures of 110-150^{o}C (Apriyantono et al., 2002; Ho et al., 2008b), while dark chocolate production normally use temperatures of 70-80^{o}C for conching process (Afoakwa, 2010). The formation of acrylamide, with asparagine as precursor (Lingnert et al., 2002; Zyzak et al., 2003), has been reported to be limited at temperatures below 200^{o}C and water content of below 30% (Lingnert et al., 2002).

Because of the increasing demand for healthier chocolate, we utilised palm sap sugar to produce a healthier chocolate which does not only contain additional minerals, vitamins, anti-oxidative and anti-carcinogenic compounds compared to standard chocolate, but might also be beneficial for people with high blood sugar levels. The aim of this work was to investigate the impact of sucrose replacement with palm sap sugar on the quality attributes of dark chocolate. Quality attributes such as colour, hardness, melting profile, flow behaviour and aroma profile were analysed and compared.

6.3 MATERIALS AND METHODS

6.3.1 Raw materials

Cocoa liquor (CM1 IC B), cocoa butter (pure prime pressed cocoa butter CB 1) and soy lecithin (SLS-578-12) were obtained from Belcolade (Erembodegem, Belgium). Coarse sucrose (Kristalsuiker EU2) was obtained from Tiense Suikerraffinaderij (Tienen, Belgium), while different kinds of coarse palm sap sugars (coconut sugar and palm sugar) were purchased from CV. Sari Nira Nusantara (Yogyakarta, Indonesia).

6.3.2 Sample preparation

Dark chocolates with a total fat content of 36% were prepared according to the following formulation: 48.0% sugar, 34.4% cocoa mass, 17.2% cocoa butter and 0.4% lecithin. Two kinds of coarse coconut sugars, namely CCS1 and CCS2, and three kinds of coarse palm sugars, namely CPS1, CPS2 and CPS3 were used as alternative sweeteners in the different chocolates. As reference sweetener, coarse sucrose (CS) was used. Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, Choc CPS3, and Choc CS were codes given for chocolates sweetened with CCS1, CCS2, CPS1, CPS2, CPS3, and CS, respectively. Specifications of the sugars can be seen in Table 6.1, with sugar composition, moisture content, protein level, and density were obtained through gas chromatography, Karl Fischer titration, Kjeldahl method, and a pycnometer, respectively.

Table 6.1. Specification of sugars used for chocolate production (dry matter basis)

Sugar	Moisture (%)	Fructose (%)	Glucose (%)	Sucrose (%)	Proteins (%)	Density (g/cm^3)
CCS1	1.8	2.4	1.2	85.0	1.2	1.52
CCS2	1.9	2.7	2.1	81.9	0.8	1.53
CPS1	1.7	1.4	0.7	84.1	1.6	1.56
CPS2	1.0	1.3	1.3	95.3	0.6	1.56
CPS3	2.4	3.3	2.0	78.7	1.3	1.54
CS	0.3	0	0	100	0	1.57

6.3.2.1 Chocolate processing

Chocolates were produced using a combination of Stephan mixing and ball milling. The production consisted of three steps, namely mixing through a Stephan Universal Machine UMC 5 (Stephan food service equipment GmbH, Hameln, Germany), refining using a ball mill (Duyvis Wiener, Koogaan de Zaan, Netherlands) and liquefaction using a Stephan mixer. The chocolate processing method was similar to the one described in Chapter 5 (Section 5.3.2.1).

6.3.2.2 Tempering and moulding

Tempering and moulding were conducted according the method described in Chapter 5 (Section 5.3.2.3).

6.3.3 Analytical methods

6.3.3.1 Moisture content

Moisture content of sugars was measured according to the method described in Chapter 3 (Section 3.3.2.1). Moisture content of chocolates was determined following the method described in Chapter 4 (Section 4.3.3).

6.3.3.2 Colour

The colour of tempered chocolate bars was measured with a colorimeter (Minolta Model CM-2500D Spectrophotometer, Tokyo, Japan) which was calibrated using a white reference standard. The SCE-mode (Specular light excluded) values were recorded and the colour parameters were expressed in terms of the CIELAB system *L** (luminance ranging from 0 (black) to 100 (white)), *a** (green to red) and *b** (blue to yellow).

6.3.3.3 Hardness

The hardness of the chocolate bars at 20 °C was evaluated with a texture analyser Instron 5942 (Norwood, MA, Canada) equipped with a needle-shaped probe with a diameter of

1 mm. The probe was set to descend with a speed of 2 mm/s and penetrated 5 mm into the bars. Hardness (N) was defined as the maximum force recorded during sample penetration.

6.3.3.4 Particle size distribution

PSD of the chocolates was evaluated according the method described in Chapter 5 (Section 5.3.3.2). D(v,0.9), D(v,0.5), D(v,0.1), D(4,3) and D(3,2), representing respectively 90%, 50% and 10% percentiles, volume-weighed mean and Sauter diameter, were derived from the recorded data. In addition, specific surface area of the particle (m^2/m^3) were also recorded.

6.3.3.5 Melting profile

Melting profile of chocolates was recorded following the method described in Chapter 5 (Section 5.3.3.3).

6.3.3.6 Flow parameters

Flow behaviour of chocolate was measured according to the method described in Chapter 4 (Section 4.3.3).

6.3.3.7 Aroma analysis

Aroma volatile profiles were analysed using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) as described in Chapter 5 (Section 5.3.3.5).

6.3.4 Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). The chocolate properties were subjected to variance analysis (ANOVA) with 5% significance level. Testing for homogeneity of variances was performed with Levene Test. When the conditions for homogeneity of variances were fulfilled, Tukey test was used to determine differences among samples. In case variances were not homogeneous, Games–Howell test was performed. Multivariate (Pearson) correlation test determined the effects of particle density and moisture content on the sensorial properties of chocolates at significant level of 1% and 5%. Principal component analysis (PCA) was used to visualise the relationships between chocolates and their volatile composition.

6.4 RESULTS AND DISCUSSION

6.4.1 Moisture content

The moisture content of ingredients is known to influence the final moisture content of chocolate, affecting its sensorial properties. Similar to fat, moisture can be present in the "bound" or "free" state within the chocolate matrix (Beckett, 2008). "Free" moisture affects the flow behaviour of chocolate to a large extent by promoting particle agglomeration. The presence of moisture in chocolate either dissolves sugar particles or creates sticky patches on the surfaces of the sugar particles, resulting in the sugar particles sticking together and an increase in flow parameter values.

Table 6.2. Comparison of moisture content of dark chocolates sweetened with different palm sap sugars and sucrose

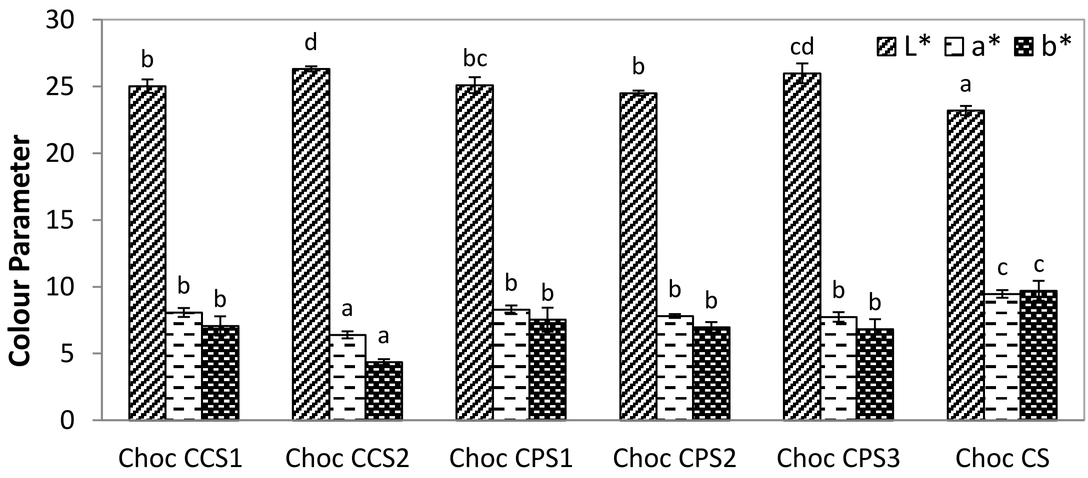
Chocolate	Moisture (%)
Choc CCS1	1.1 ± 0.01 [c]
Choc CCS2	1.2 ± 0.01 [c]
Choc CPS1	1.1 ± 0.07 [c]
Choc CPS2	0.8 ± 0.02 [b]
Choc CPS3	1.5 ± 0.00 [d]
Choc CS	0.6 ± 0.02 [a]

Mean values ± standard deviations
Different superscripts indicate significant differences ($p < 0.05$) among samples

The results (Table 6.2) showed that dark chocolates sweetened with the palm sap sugars recorded significantly ($p < 0.05$) higher moisture levels than the chocolate sweetened with sucrose. This can be attributed to the initial moisture content of the alternative sweeteners and presence of hygroscopic materials, *e.g.,* glucose and fructose (Table 6.1), resulting in a higher chocolate moisture level.

6.4.2 Colour

Colour is one of the visual attributes used to describe the appearance of chocolate aside from gloss, shape, surface, and smoothness or roughness (Briones et al., 2006). Colour of chocolate is not only influenced by processing methods, but also by composition and roughness of the chocolate surface. Palm sap sugars recorded a somewhat lower particle density than sucrose (Table 6.1), resulting in a higher particle volume fraction and, hereby, increasing particle-particle interactions. This is likely to result in a more denser chocolate, able to scatter more light resulting in a lighter colour. Afoakwa et al (2008e, f) also drew similar conclusions when comparing the influence of different particle sizes on the colour of dark chocolate. They mentioned that chocolates with fine particles (18–25 µm) have more particle-particle interactions and, thus, appear lighter and are more saturated than chocolate with coarser particles (35–50 µm). It can be observed from Figure 6.1 that chocolates sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, and Choc CPS3) exhibited significantly higher *L** values ($p < 0.05$) than chocolate sweetened with sucrose (Choc CS). Among the chocolates sweetened with palm sap sugar, Choc CCS2 exhibited a significantly ($P<0.05$) lower *a** and *b** value but showed a rather similar *L** value compared to the others. This observation might be attributed to the smoothness/roughness of the chocolate surface. Chocolate with a different level of surface roughness will scatter light distinctively, resulting in a different colour perception by the human eye and colorimeter. Briones et al. (2006), using digital vision system to observe the effect of surface topography on colour and gloss of chocolate samples, reported that chocolate with smooth surface has higher *L** value indicating a lighter colour.



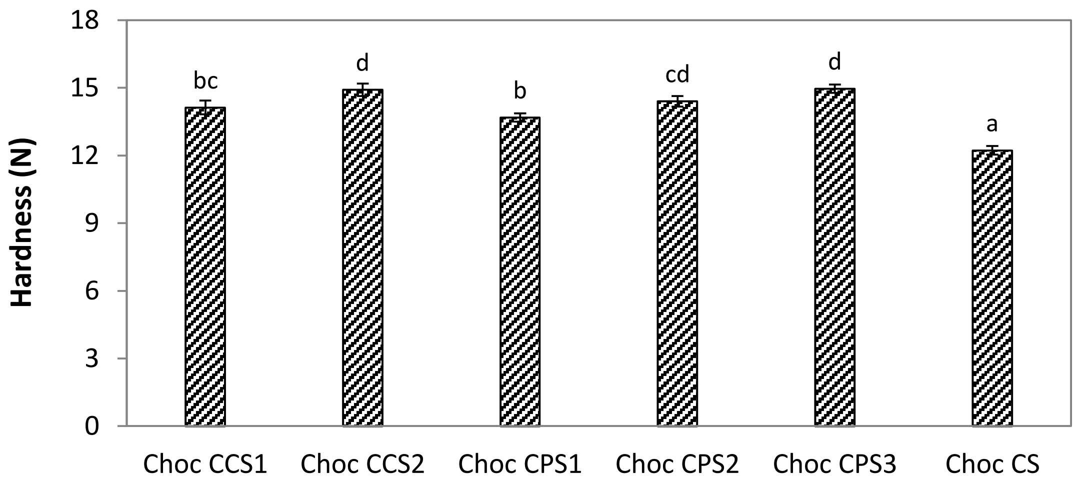
Different superscripts per parameter indicate significant differences ($p < 0.05$) among samples

Figure 6.1. Comparison of colour parameters of chocolates formulated with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, Choc CPS3) and sucrose (Choc CS)

It was previously hypothesised that the presence of reducing sugars (glucose and fructose) and proteins in palm sap sugar (Table 6.1) could induce additional Maillard reactions which might intensify the darkness of the chocolates. Thus, it was expected that the colour of chocolates sweetened with palm sap sugar would be darker. On the contrary, it resulted in chocolates with lighter colour compared to that of chocolate sweetened with sucrose. It seemed that the low particle density of palm sap sugar, which create more particle-particle interaction, gave more influence to the colour formation of chocolate than Maillard reaction. Although the colour of palm sap sugars were darker than the colour of sucrose, it did not influence the darkness of the chocolates. It can be observed in Table 6.6 that particle density was inversely correlated to lightness of chocolate.

6.4.3 Hardness

Chocolate hardness is a quality parameter commonly affected not only by fat content but also by particle volume fraction, particle size distribution (Afoakwa et al., 2008e) and the tempering process (Afoakwa, 2010; Shah et al., 2010). Chocolate sweetened with sucrose (Choc CS) exhibited a significantly lower hardness ($p < 0.05$) than those sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, and Choc CPS3) (Figure 6.2). This can be attributed to differences in the particle density, particle size distribution, moisture content, and sugar composition of sweeteners. The incorporation of palm sap sugars, exhibiting lower density than sucrose, led to an increased particle volume fraction resulting in increased particle-particle interactions and thus, harder chocolate. Afoakwa et al (2008e, f) reported that chocolates with fine particles (18–25 µm) which have more particle-particle interactions resulting in harder texture than chocolates with coarser particles (35–50 µm). Do et al (2007), based on their finding in the impact of PSD on rheological and textural properties of chocolate models with reduced fat content, also stated that a reduced hardness can be based on the decreased contact levels between particles in suspension. Chocolates formulated with palm sap sugars also showed significantly ($p < 0.05$) higher moisture levels than sucrose-sweetened chocolate (Table 6.2) which could have contributed to the increased hardness of chocolates formulated with palm sap sugars. The presence of high moisture content due to inadequate moisture evaporation during palm sap sugar production might have created more amorphous sugar in chocolates leading to the formation of "strong" sugar networks in chocolate (Beckett, 2009; Stortz and Marangoni, 2011). Aside from this, the presence of fructose and glucose as hygroscopic materials might also have created more sugar networks, resulting in a higher force required to penetrate chocolate, explaining to some extent the high hardness values recorded for chocolates formulated with palm sap sugars. It can be observed in Table 6.6 that hardness had negative and positive significant correlation ($p < 0.05$) to particle density and moisture, respectively. Among the chocolates sweetened with palm sap sugar, Choc CCS2 and Choc CPS3 exhibited a significantly higher ($P<0.05$) hardness compared to the others. This observation might be attributed to a higher moisture content of chocolates (Table 6.2) and a lower particle densities of the sugars used for the chocolate production (Table 6.1). A slight difference in hardness was observed in the rest of chocolates formulated with palm sap sugars. This observation might be attributed to the different concentration of chemical "impurities" in palm sap sugar such as proteins, reducing sugar and minerals which influence the particle-particle interaction and sugar network in chocolate matrix.



Different superscripts indicate significant differences ($p < 0.05$) among samples

Figure 2.2. Comparison of hardness of chocolates formulated with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, Choc CPS3) and sucrose (Choc CS)

All the factors previously mentioned, namely particle density, particle size distribution, moisture content, and sugar composition can be used to evaluate the hardness of chocolate in case it is well-tempered. Shah et al. (2010) reported that degree of tempering affect the hardness of chocolate. A well-tempered chocolate with low particle density is supposed to have a higher hardness. However, in their finding, chocolate containing inulin HPX which has low density, exhibited the lowest hardness due to poor degree of tempering. Nevertheless, in our study, all chocolates were well tempered, which was verified using temper meter (see Section 6.3.2.2), thus the differences of chocolate hardness in this experiment was not related to the degree of tempering.

6.4.4 Particle size distribution

PSD is a key determinant of the flow properties and sensory perception of chocolates. Smaller particles improve the sensory properties, whereas, plastic viscosity and yield stress increase due to the increase in specific surface area of the dispersed particles (Ziegler et al., 2001). In order to obtain high-quality chocolate, PSD optimisation should consider palate sensitivity. Chocolate with large particles (> 30 µm) is perceived as 'gritty' , while particle sizes of 20 µm creates a creamier taste and texture (Afoakwa, 2010; Do et al., 2007).

In this work, PSD measurement was carried out following two different sample preparations: with and without sonication (Table 6.3). As mentioned previously, high moisture and the presence of fructose and glucose as hygroscopic materials might create the presence of more amorphous part during chocolate production, leading to particle agglomeration. The sonication pre-treatment aimed to break the particle agglomeration in order to measure the actual size of the dispersed particles. Chocolates with high degree of agglomeration due to a higher moisture level and the presence of fructose and glucose exhibited higher reduction of particle size (after application of sonication pre-treatment) than chocolates with low degree of agglomeration, depicting the significant impact of the sonication process. Aside from this, the presence of agglomeration can be observed from the melting temperature of sugar in chocolate. The presence of relatively higher moisture, which initiate particle agglomeration, can be confirmed with a lower melting point and enthalpy of sugar in chocolate as compared to that of chocolate with low moisture content (Figure 6.3 and Table 6.5).

Table 6.3. Comparison of particle size distribution parameters of non-sonicated and sonicated chocolates sweetened with palm sap sugars and sucrose

Chocolate	PSD (without Sonication pre-treatment)					
	Distribution Percentiles (µm)			Derived Diameter (µm)		Specific Surface Area (m^2/m^3)
	D(v,0.9)	D(v,0.5)	D(v,0.1)	D(4,3)	D(3,2)	
Choc CCS1	46,7 ± 0,6 ^{d}	9,7 ± 0,1 ^{c}	1,4 ± 0,0 ^{c}	17,8 ± 0,2 ^{c}	2,6 ± 0,1 ^{c}	2.4 ± 0.1 ^{b}
Choc CCS2	46,0 ± 0,2 ^{d}	9,7 ± 0,1 ^{c}	1,4 ± 0,0 ^{c}	17,6 ± 0,1 ^{c}	2,6 ± 0,0 ^{bc}	2.3 ± 0.2 ^{b}
Choc CPS1	31,4 ± 0,6 ^{c}	6,4 ± 0,0 ^{b}	1,2 ± 0,0 ^{b}	12,0 ± 0,1 ^{b}	2,3 ± 0,0 ^{ba}	2.6 ± 0.1 ^{bc}
Choc CPS2	20,4 ± 0,7 ^{b}	5,7 ± 0,1 ^{a}	1,2 ± 0,0 ^{bc}	8,7 ± 0,3 ^{a}	2,2 ± 0,1 ^{a}	2.7 ± 0.1 ^{c}
Choc CPS3	57,3 ± 0,6 ^{e}	13,0 ± 0,2 ^{d}	1,8 ± 0,0 ^{d}	22,6 ± 0,3 ^{d}	3,1 ± 0,1 ^{d}	1.9 ± 0.1 ^{a}
Choc CS	18,2 ± 1,0 ^{a}	5,5 ± 0,2 ^{a}	1,1 ± 0,0 ^{a}	8,1 ± 0,4 ^{a}	2,2 ± 0,2 ^{a}	2.7 ± 0.0 ^{c}

Chocolate	PSD (with Sonication pre-treatment)					
	Distribution Percentiles (μm)			Derived Diameter (μm)		Specific Surface Area (m^2/ m^3)
	D(v,0.9)	D(v,0.5)	D(v,0.1)	D(4,3)	D(3,2)	
Choc CCS1	20,8 ± 0,1 ^{bcd}	6,1 ± 0,2 ^{bc}	1,1 ± 0,0 ^{ac}	8,8 ± 0,1^{c}	2,2 ± 0,1 ^{ab}	2.7 ± 0.1 ^{b}
Choc CCS2	19,3 ± 0,5 ^{bc}	6,6 ± 0,2 ^{c}	1,4 ± 0,0 ^{b}	8,7 ± 0,2^{c}	2,6 ± 0,1 ^{b}	2.4 ± 0.0 ^{a}
Choc CPS1	18,9 ± 0,7 ^{bc}	5,6 ± 0,3 ^{ab}	1,1 ± 0,1 ^{a}	8,1 ± 0,4^{b}	2,1 ± 0,1 ^{a}	2.9 ± 0.1 ^{c}
Choc CPS2	14,9 ± 0,3 ^{a}	5,2 ± 0,2 ^{a}	1,1 ± 0,1 ^{a}	6,9 ± 0,2^{a}	2,0 ± 0,1 ^{a}	3.0 ± 0.1 ^{c}
Choc CPS3	25,9 ± 1,1 ^{cd}	7,9 ± 0,3 ^{d}	1,4 ± 0,2 ^{bc}	11,2 ± 0,2^{d}	2,6 ± 0,3 ^{b}	2.4 ± 0.3 ^{a}
Choc CS	15,9 ± 0,3 ^{a}	5,4 ± 0,1 ^{a}	1,2 ± 0,1 ^{abc}	7,3 ± 0,2^{a}	2,3 ± 0,1 ^{ab}	2.6 ± 0.0 ^{b}

Mean values ± standard deviations
Different superscripts per parameter indicate significant differences ($p < 0.05$) among samples

The results show that a lot of sugar particle agglomeration occurred in the chocolates sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, and Choc CPS3), in contrast to the chocolate sweetened with sucrose (Choc CS). Based on D(v,0.9) value, it can be observed that the higher the moisture content of the chocolate, the greater the effect of the sonication process on the de-agglomeration of the solid particles. It can be observed in Table 6.6 that the moisture content of chocolate is highly correlated to the D(v,0.9) value measured without sonication pre-treatment.

6.4.5 Melting profile

Cocoa butter can crystallise in different polymorphs, with type I being the least stable, type V the most desirable and type VI the most stable crystal form. The latter can be obtained from the crystal V transformation during storage. Type V crystal with a melting point of 32-34^{o}C can be achieved by means of tempering which enhances chocolate gloss, snap and shelf life (Afoakwa et al., 2008d; Beckett, 2009; Fernandes et al., 2013; Glicerina et al., 2013; Keijbets et al., 2010).

The DSC profile of chocolates were measured from 20^{o}C to 200^{o}C and showed 2 endothermic transitions (Figure 6.3). The first peak can be attributed to fat melting (cocoa butter), which corresponds to the melting behaviour of chocolate during consumption, while the second peak can be associated with sugar melting/degradation. The latter is not directly related to sensorial properties, however, the insight of sugar melting in chocolate can be used to understand more about the presence of amorphous sugar and/or moisture.

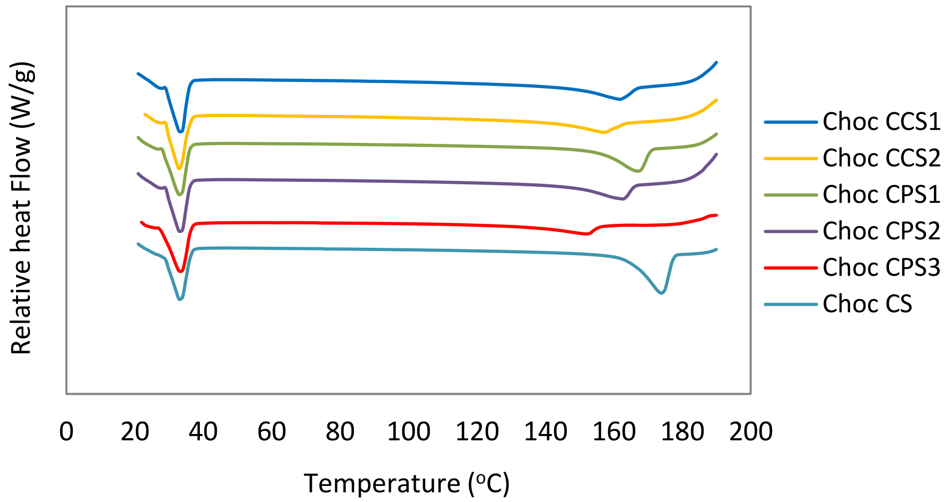


Figure 6.3. Comparison of melting profile of chocolates sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, Choc CPS3) and sucrose (Choc CS)

With regard to fat melting, it was observed that chocolates sweetened with palm sap sugar and sucrose exhibited similar peak maximum temperature and width (Table 6.4). Nevertheless, Choc CPS1 and Choc CPS3 exhibited slightly lower onset temperature than that of Choc CCS1, Choc CCS2, Choc CPS2 and Choc CS which might be attributed to the differences in particle sizes, moisture content, and sugar compositions leading to different breakage and melting temperature. Moreover, different thermal contact between the sample and the DSC pan at the beginning of melting measurement, might result in different onset temperature. It can also be observed that the enthalpy value, as seen in Table 6.4, varied among samples, which was associated with the different energy required to complete the melting of fat.

Table 6.4. Comparison of the fat melting parameters of chocolates sweetened with palm sap sugars and sucrose

Chocolate	Onset (°C)	Peak Max (°C)	Width (°C)	Enthalpy (J/g)
Choc CCS1	29,3 ± 0,1 ^{b}	33,8 ± 0,1 ^{a}	4,1 ± 0,4 ^{a}	28,0 ± 0,2 ^{a}
Choc CCS2	29,0 ± 0,1 ^{b}	33,5 ± 0,2 ^{a}	4,3 ± 0,8 ^{a}	29,4 ± 0,9 ^{ab}
Choc CPS1	28,3 ± 0,2 ^{a}	33,5 ± 0,3 ^{a}	4,4 ± 0,4 ^{a}	31,6 ± 0,3 ^{bc}
Choc CPS2	29,2 ± 0,0 ^{bc}	33,8 ± 0,1 ^{a}	4,2 ± 0,4 ^{a}	28,3 ± 0,3 ^{a}
Choc CPS3	27,5 ± 0,4 ^{ac}	33,7 ± 0,3 ^{a}	4,8 ± 0,2 ^{a}	33,4 ± 2,4 ^{c}
Choc CS	29,1 ± 0,1 ^{b}	33,6 ± 0,2 ^{a}	4,3 ± 0,4 ^{a}	28,0 ± 0,4 ^{a}

Mean values ± standard deviations
Different superscripts per parameter indicate significant differences ($p < 0.05$) among samples

The melting ranges of the sugar phase seemed highly depending on the presence of water as plasticiser (Beckett et al., 2006; Kedward et al., 1998b; Kedward et al., 2000). It can be observed in Table 6.5 and Figure 6.3 that chocolates formulated with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, Choc CPS3) exhibited lower onset and peak temperatures compared to the one sweetened with sucrose (Choc CS). The fact that palm sap sugars contain more moisture than sucrose might have triggered the development of more amorphous matter during chocolate production. Glicerina et al (2013) stated that small amounts of amorphous sugar can be obtained after the absorption of moisture by sugar particles. It can be seen in Table 6.6 that moisture content is inversely correlated to the melting peak temperature and also enthalpy. Moreover, the presence of “impurities” (protein, minerals, and reducing sugar) in the palm sap sugars may have contributed to the lowering of the onset and peak temperature of palm sap sugar chocolates. It was stated that the reduction of the melting point of a substance can be correlated to the presence of impurities (Beckett et al., 2006).

Table 6.5. Comparison of sugar melting parameters of chocolates sweetened with palm sap sugars and sucrose

Chocolate	Onset (°C)	Peak Max (°C)	Width (°C)	Enthalpy (J/g)
Choc CCS1	150,6 ± 0,8 ^{c}	164,2 ± 0,5 ^{bd}	9,8 ± 0,1 ^{cd}	20,4 ± 1,6 ^{b}
Choc CCS2	144,0 ± 2,6 ^{b}	158,4 ± 2,0 ^{abd}	9,9 ± 0,5 ^{ce}	17,4 ± 1,0 ^{b}
Choc CPS1	155,9 ± 0,7 ^{d}	167,5 ± 0,4 ^{c}	8,8 ± 0,4 ^{b}	32,1 ± 0,9 ^{c}
Choc CPS2	148,1 ± 0,4 ^{c}	162,8 ± 0,3 ^{bd}	10,5 ± 0,2 ^{d}	23,5 ± 1,3 ^{b}
Choc CPS3	135,6 ± 0,2 ^{a}	152,3 ± 0,2 ^{ab}	11,5 ± 0,3 ^{e}	14,6 ± 0,5 ^{a}
Choc CS	164,8 ± 0,8 ^{e}	174,1 ± 0,5 ^{e}	7,5 ± 0,3 ^{a}	47,1 ± 1,4 ^{d}

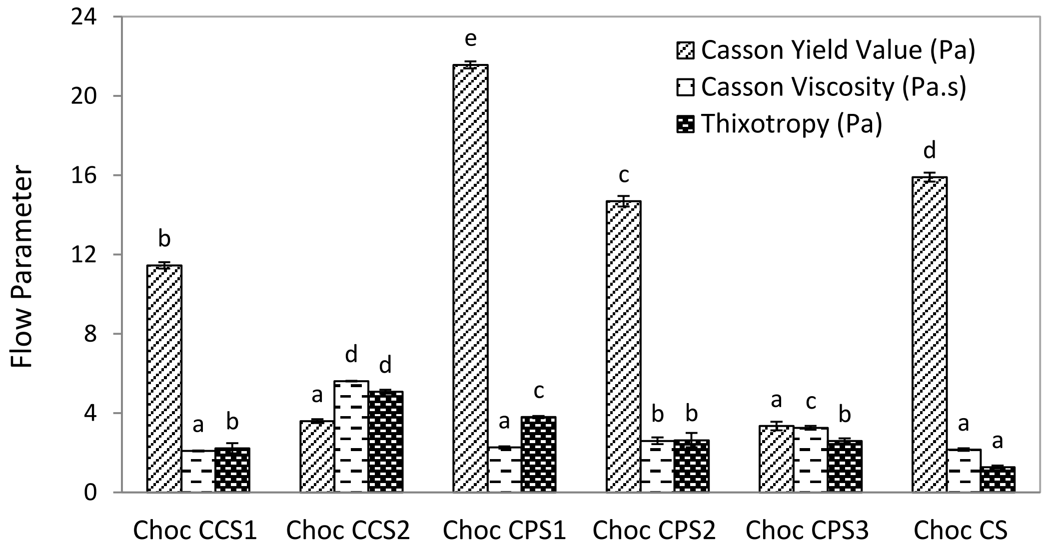
Mean values ± standard deviations
Different superscripts per parameter indicate significant differences ($p < 0.05$) among samples

It can be seen in Table 6.5 that palm sap sugar chocolates (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, Choc CPS3) also exhibited significantly lower ($p < 0.05$) sugar melting enthalpies than the one sweetened with sucrose (Choc CS). This can be attributed to the presence of more moisture and “impurities” in the palm sap sugar. Samples with high moisture and presence of “impurities” will exhibit less crystalline part of sugar resulting in lower enthalpy needed, indicated by a blunt, broad and wide melting curve, as compared to “pure” samples low in moisture, resulting in a higher enthalpy needed and indicated by a sharp, narrow melting curve (Figure 6.3). The presence of impurities and additives (including mixed sugar systems) affects both the nucleation and growth steps (Hartel and Shastry, 1991). During crystal formation, sugar with low purity has fewer number of nuclei compared to that of sugar with high purity.

6.4.6 Flow behaviour

Flow properties of chocolates are very important, not only with regard to mouthfeel and consumer acceptance but also for the handling properties during application. Rheologically, molten chocolate exhibits a non-Newtonian flow behaviour which is usually described by a yield stress and plastic viscosity. The former is defined as the minimum stress to initiate flow and determined by the absolute distance between solid particles in suspension (Afoakwa et al., 2007). The results showed that Choc CCS1, Choc CPS1, Choc CPS2 and Choc CS exhibited significantly higher yield values ($p < 0.05$) than Choc CCS2 and Choc CPS3 (Figure 6.4). This phenomenon can be attributed to their D(v,0.9) and D(3,2) value of the dispersed solids. Based on the PSD measurement (Table 6.3), Choc CCS2 and Choc CPS3 exhibited relatively higher D(v,0.9) and D(3,2) values than the others (Choc CCS1, Choc CPS1, Choc CPS2, and Choc CS) which implies that Choc CCS2 and Choc CPS3 had larger particles and a lower specific surface areas resulting in less particle-particle interactions. Mongia et al (2000) stated that at low shear stresses, the flow behaviour is mainly dominated by particle-particle interactions. The smaller the particle size, the larger the specific surface area, thus, the stronger the particle-particle interaction will be, resulting in higher yield values. It also can be seen in Figure 6.4 that Choc CCS1 exhibited a higher yield value compared to Choc CCS2 and Choc CPS3. This observation might be attributed to the fact that Choc CCS1 exhibited a higher specific surface area (Table 6.3), a higher particle volume fraction due to a lower sugar density (Table 6.1) and/or a lower reducing sugar and moisture content compared to Choc CCS2 and Choc CPS3 (Table 6.1 and Table 6.2). Chocolate with a lower moisture, fructose and glucose content creates a lower degree of agglomeration, resulting in a higher surface area, leading to a higher yield value. It can be observed in Table 6.6 that yield value was inversely correlated to the moisture and reducing sugar (*e.g.* glucose and fructose) content.

The Casson viscosity represents the internal friction of the chocolate determining coating thickness amongst others (Beckett, 2008). The results showed (Figure 6.4) that Casson viscosity of the samples were influenced by particle density and moisture content of the chocolates. Choc CS which exhibited the lowest moisture content and the highest particle density had the lowest Casson viscosity. Conversely, Choc CCS2 with relatively high moisture content and low particle density, exhibited the highest Casson viscosity.



Different superscripts per parameter indicate significant differences ($p < 0.05$) among samples

Figure 6.4. Comparison of flow properties of chocolates sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS 2, Choc CPS3) and sucrose (Choc CS)

It can be observed in Figure 6.5 that PSD curves of Choc CCS 1, Choc CCS2, Choc CPS1 and Choc CPS3 measured without sonication pre-treatment exhibited bimodal particle size distribution, while Choc CPS2 and Choc CS exhibited unimodal particle size distribution (Figure 6.5A). However, unimodal PSD curves were observed in all chocolates when sonication pre-treatment prior to PSD measurement was used (Figure 6.5B). This implied that bimodal PSD curves were formed due to the presence of particle agglomeration. Nevertheless, during shearing, the agglomerated particles might be fragmented, changing the initial packing fraction of solid particles, altering the particle-particle interaction. Due to this phenomenon, the influence of modality of particle size distribution in determining the viscosity of chocolates was less pronounced than the effect of particle density and moisture content.

Thixotropy can be used to evaluate the presence of agglomeration, one of indicators for unwell-conched chocolate. Well-conched chocolates should not exhibit thixotropic behaviour (Servais et al., 2004). The results showed that chocolates sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, and Choc CPS3) exhibited significantly higher thixotropy ($p < 0.05$) than that sweetened with sucrose (Choc CS). This was explained by the presence of high moisture content in the chocolates sweetened with palm sap sugars creating agglomeration of the particles, hence resulting in a time-dependent flow behaviour.

Table 6.6 . Correlation between chocolate sensorial properties and particle density of sugars, reducing sugar, moisture content of sugar and chocolate

Parameter	Particle density (g/cm^3)	Reducing sugar (%)	Moisture Content (%)	
			Sugar	Chocolate
PSD				
D(v,0,9) without sonication	-0.786**	0.893*	0.907**	0.953**
D(v,0,9) with sonication	-0.590**	0.742*	0.833**	0.941**
Colour				
L*	-0.719**	0.845*	0.913**	0.843**
a*	0.657**	-0.788*	-0.675**	-0.518*
b*	0.614**	-0.707*	-0,642**	-0,485*
Hardness	-0.605**	0.919**	0.813**	0.703*
Flow properties				
Casson yield value (σ_{CA}) (Pa)	0.698**	-0.818**	-0.565**	-0.626**
Casson viscosity (η_{CA}) (Pa.s)	-0.526**	0.609**	0.435*	0.372
Thixotropy (Pa)	-0.419	0.514*	0.566*	0.395
T_{max} of sugar in chocolate (^oC)	0.631**	-0.951**	-0.839**	-0.812**
Enthalpy of sugar melting (J/g)	0.693**	-0.959**	-0.853**	-0.754**

*significant at $p < 0.01$, ** significant at $p < 0.05$

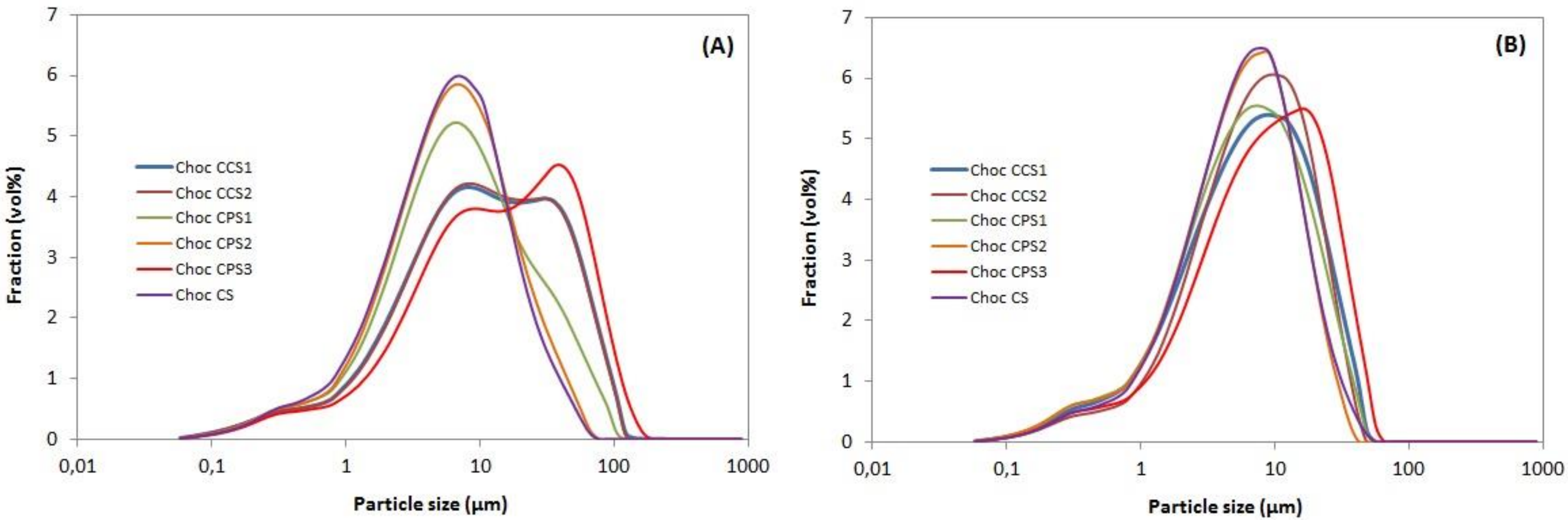


Figure 6.5. PSD curves of chocolate sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, and Choc CPS3) and sucrose (Choc CS). (A) PSD curve of chocolate measured without sonication pre-treatment, (B) PSD curve of chocolate measured with sonication pre-treatment.

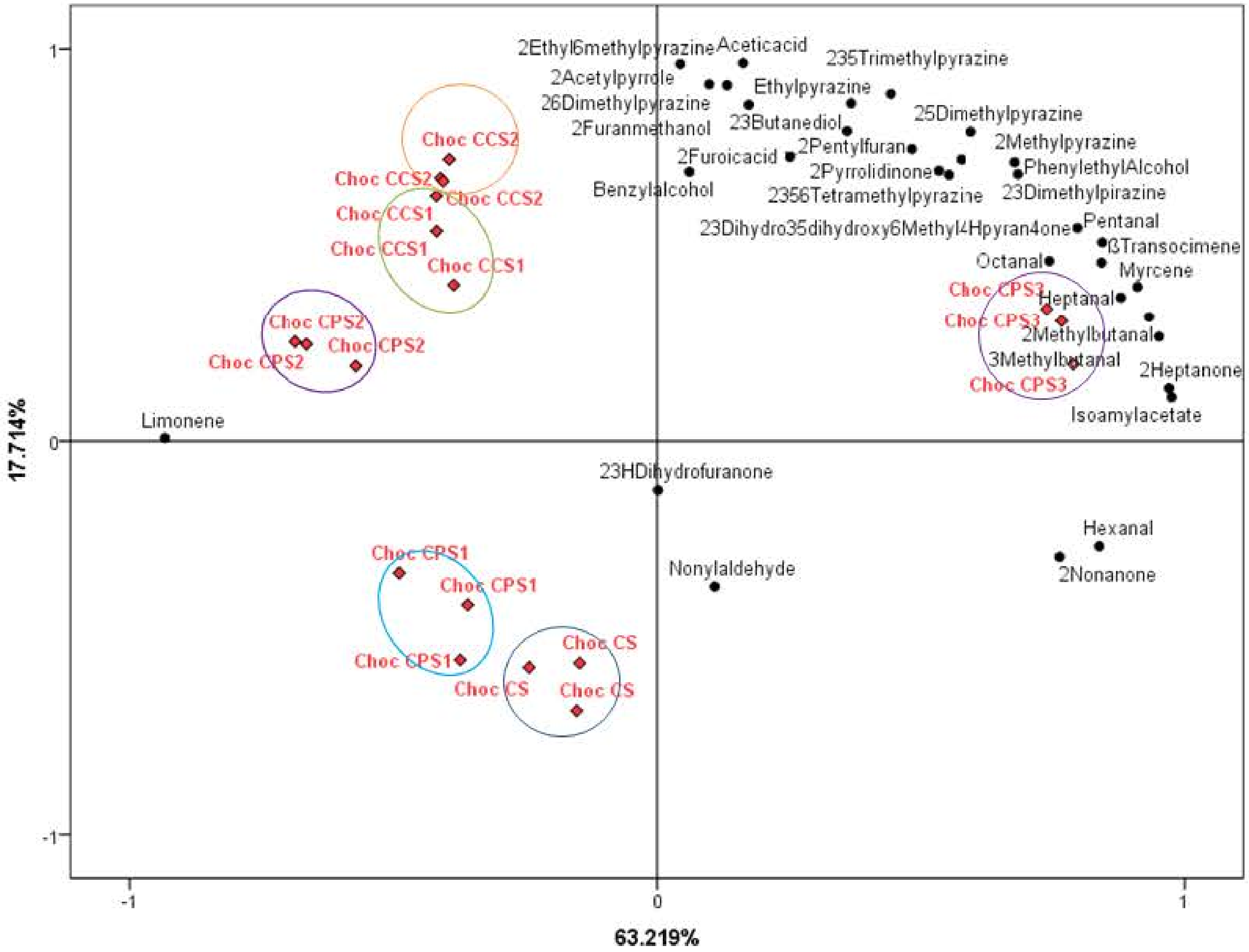


Figure 6.6. PCA (loadings and score plot) of aroma volatiles in chocolates sweetened with palm sap sugars (Choc CCS1, Choc CCS2, Choc CPS1, Choc CPS2, Choc CPS3) and sucrose (Choc CS)

6.4.7 Aroma profile

Pure sucrose, the most common chocolate sweetener, is not considered as a factor influencing the aroma formation of the chocolate. Beckett (1999) stated that sugar is considered as an inert ingredient in chocolate, contributing ‘’only’’ to sweetness. However, the usage of alternative sugars might also be an influential factor for chocolate aroma. Since palm sap sugars contain in addition to sucrose, proteins and reducing sugars (BPS, 2010; BSN, 1995; Phaichamnan et al., 2010a), heat applied during the production of chocolate with palm sap sugars as sweetener might induce additional Maillard reactions. This might lead to the development of a deviating, distinctive chocolate aroma. Aside from this, some volatiles which are present in palm sap sugar are likely to also appear in palm sap sugar chocolate.

PCA data analysis of aroma volatiles was demonstrated in Figure 6.6. PCA explained ~ 81% variance in the first two factors, with PC1: 63.2% and PC2: 17.7%. In general, it can be observed that there were 3 clusters of chocolate. The first cluster (Choc CPS3), characterised by high positive values in PC1, exhibited high aroma volatile concentrations of isoamyl acetate, 2-heptanone, 3-methyl butanal, 2-methyl butanal, myrcene and pyrazine-based compounds. The second cluster (Choc CCS 1, Choc CCS2, and Choc CPS2) contained high aroma volatile concentration, namely 2-ethyl-6-methylpyrazine, acetic acid, 2-acetylpyrrole, 2,6-dimethylpyrazine, 2,3,5-trimethylpyrazine, ethylpyrazine which were characterised by high positive value in PC2. As third cluster, it was observed that a chocolate sweetened with palm sap sugar, Choc CPS1, was closely clustered to that sweetened with sucrose (Choc CS), which contained low pyrazine based compounds, but rather high of nonylaldehyde and 2(3H)-dihydrofuranone.

Due to different physicochemical characteristic of palm sap sugar as indicated by different moisture, glucose, fructose, sucrose, protein content and also particle density (Table 6.1), it was still understandable that palm sap sugar sweetened chocolates were not in the same cluster. Aside from this, aroma volatiles composition of palm sap sugars as chocolate sweetener, which might be different among sugars, seemed to play very important role on the aroma volatiles observed in the chocolates. In general, the key aroma volatiles of chocolates sweetened with palm sap sugar and sucrose can be seen in Table 6.7. It can be observed that compared to the reference (Choc CS), chocolates formulated with palm-sap based sugar (Choc CCS1, Choc CCS2, Choc CPS2, and Choc CPS3) exhibited significantly higher ($p < 0.05$) concentration of pyrazine-based compounds, which generally confer roasted/caramel-like aroma and some other volatiles such as 2(3H)-dihydrofuranone, 2-furan methanol, 2,3-butanediol, and 2-acetylpyrrole which confer sweet, nutty, popcorn, and caramel-like aroma than chocolate sweetened with sucrose (Choc CS) (Afoakwa et al., 2009a; Owusu et al., 2012; Ramos et al., 2014; Rodriguez-Campos et al., 2012; Tran et al., 2015a). The fact that palm sap sugars also contain acetic acid (Apriyantono et al., 1996; Ho et al., 2006b; Purnomo, 2007), derived from the coconut/palm sap as raw material for sugar production, might be the reason for significantly higher ($p < 0.05$) acetic acid compounds in palm sap sugar sweetened chocolates than that in sucrose sweetened chocolate. Aside from this, a higher amorphous part in palm sap sugar sweetened chocolate might increase the concentration of acetic acid and other aroma volatiles observed in the chocolates. Beckett (2008) stated that if sugar is milled together with cocoa, some of the volatile cocoa flavours are absorbed by the amorphous sugar, resulting in a more intense flavour chocolate.

Moreover, it can also be seen in Table 6.7 that palm sap sugar chocolates also contained high amounts of 2,3‑dihydro‑3,5‑dihydroxy‑6‑methyl‑4(H)‑pyran‑4‑one (DDMP) and 2-furoic acid which were absent in chocolate sweetened with sucrose (Choc CS). Since pyrazine based compounds and DDMP are also present in palm sap sugar (Ho et al., 2006b), the different concentration of pyrazine based compounds and the presence of DDMP can be associated to the presence of those compounds in palm sap sugars and/or due to the additional Maillard reaction during chocolate production. Nevertheless, since the browning effect of Maillard reactions did not influence the darkness of palm sap sugar sweetened chocolates, it seems that Maillard reaction occurred in a minimal extent during chocolates production, so that the distinctive aroma volatiles in palm sap sugars sweetened chocolate mainly resulted from the palm sap sugars volatiles. Hoskin and Dimick (1983) reported that during conching, temperature applied and/or the concentrations of free amino acids and sugars would be too low for Maillard reactions. Aside from this, the moisture content of chocolate is relatively low, thus the mobility of the reactants is very limited, leading to slow rate of Maillard reaction.

Table 6.7. Mean semi-quantitative concentrations of aroma volatiles (ng/g) in chocolates sweetened with palm sap sugars and sucrose identified by HS-SPME-GC-MS

Aroma Volatiles	Concentration (ng/g)					
	Choc CCS1	Choc CCS2	Choc CPS1	Choc CPS2	Choc CPS3	Choc CS
a. Pyrazines						
Methylpyrazine	47.3 ± 3.4 ^{c}	58.7 ± 3.1 ^{cd}	10.0 ± 1.2 ^{a}	34.7 ± 3.3 ^{b}	66.1 ± 3.3 ^{f}	29.7 ± 2.9 ^{b}
2,3-Dimethylpirazine	21.6 ± 2.0 ^{bc}	26.5 ± 5.8 ^{bc}	11.6 ± 3.7 ^{a}	18.3 ± 3.1 ^{ab}	29.6 ± 1.9 ^{c}	17.3 ± 3.0 ^{ab}
2,5-Dimethylpyrazine	60.7 ± 0.2 ^{c}	69.0 ± 1.4 ^{d}	30.1 ± 0.9 ^{a}	40.5 ± 1.7 ^{b}	81.0 ± 0.2 ^{f}	30.7 ± 1.0 ^{a}
2,6-Dimethylpyrazine	86.2 ± 3.0 ^{e}	92.5 ± 1.4 ^{f}	15.5 ± 0.6 ^{b}	28.9 ± 2.2 ^{c}	62.8 ± 2.7 ^{d}	12.2 ± 0.3 ^{a}
Ethylpyrazine	16.9 ± 1.6 ^{c}	20.0 ± 0.2 ^{d}	10.7 ± 1.1 ^{a}	13.7 ± 0.7 ^{b}	18.6 ± 0.1 ^{cd}	11.9 ± 0.8 ^{a}
2,3,5-Trimethylpyrazine	89.9 ± 3.5 ^{c}	95.6 ± 1.8 ^{cd}	63.5 ± 2.6 ^{a}	80.9 ± 3.0 ^{b}	101.0 ± 1.4 ^{d}	63.9 ± 5.0 ^{a}
2,3,5,6-Tetramethylpyrazine	361.9 ± 12.2 ^{b}	379.5 ± 6.9 ^{b}	294.2 ± 18.8 ^{a}	331.6 ± 9.4 ^{ab}	435.3 ± 24.2 ^{c}	312.0 ± 24.7 ^{a}
2-Ethyl-6-methylpyrazine	16.6 ± 0.9 ^{c}	16.7 ± 1.3 ^{c}	6.5 ± 1.6 ^{a}	11.2 ± 0.3 ^{b}	13.2 ± 0.9 ^{b}	6.4 ± 0.3 ^{a}
2-Pyrrolidinone	29.0 ± 4.8 ^{b}	27.9 ± 5.9 ^{b}	19.0 ± 2.8 ^{b}	25.0 ± 1.3 ^{b}	43.5 ± 7.5 ^{c}	6.1 ± 1.1 ^{a}
b. Aldehydes						
2-Methyl butanal	11.5 ± 0.4 ^{b}	11.1 ± 0.4 ^{b}	7.5 ± 1.1 ^{a}	7.7 ± 0.2 ^{a}	29.3 ± 0.8 ^{c}	7.8 ± 2.0 ^{a}
3-Methyl butanal	28.7 ± 1.6 ^{ab}	33.9 ± 1.1 ^{b}	23.9 ± 1.2 ^{a}	22.3 ± 0.8 ^{a}	75.0 ± 2.4 ^{c}	26.4 ± 4.8 ^{a}
Pentanal	24.3 ± 1.2 ^{b}	29.7 ± 0.8 ^{c}	17.1 ± 0.5 ^{a}	17.2 ± 1.1 ^{a}	45.6 ± 1.2 ^{d}	16.8 ± 1.5 ^{a}
Hexanal	56.9 ± 0.4 ^{bc}	47.2 ± 0.4 ^{a}	53.9 ± 03 ^{b}	42.6 ± 1.6 ^{a}	68.0 ± 2.7 ^{d}	60.7 ± 2.5 ^{c}
Heptanal	12.8 ± 0.9 ^{cd}	14.3 ± 0.4 ^{c}	10.6 ± 0.4 ^{bc}	8.5 ± 0.3 ^{a}	20.5 ± 2.0 ^{d}	11.1 ± 0.7 ^{bc}
Octanal	41.8 ± 5.2 ^{b}	42.6 ± 2.7 ^{b}	39.1 ± 7.4 ^{b}	32.4 ± 6.7 ^{b}	67.1 ± 1.4 ^{c}	23.5 ± 0.5 ^{a}
Nonylaldehyde	46.9 ± 18.5 ^{a}	57.5 ± 1.7 ^{a}	68.7 ± 18.3 ^{a}	48.7 ± 29.0 ^{a}	58.5 ± 2.6 ^{a}	49.8 ± 5.6 ^{a}
Benzaldehyde	30.4 ± 4.4 ^{b}	61.9 ± 5.6 ^{c}	82.9 ± 15.1 ^{d}	19.6 ± 0.3 ^{a}	172.3 ± 9.7 ^{f}	106.7 ± 28.5 ^{e}
c. Furans						
2-Pentylfuran	29.5 ± 1.9 ^{b}	32.9 ± 2.9 ^{b}	21.7 ± 0.5 ^{a}	24.2 ± 0.7 ^{a}	32.5 ± 1.4 ^{b}	24.9 ± 1.3 ^{a}
2(3H)-Dihydrofuranone	99.7 ± 5.1 ^{c}	116.3 ± 1.5 ^{e}	50.2 ± 0.7 ^{b}	108.1 ± 4.5 ^{d}	131.9 ± 4.2 ^{f}	41.7 ± 2.6 ^{a}
2-Furan methanol	127.2 ± 2.3 ^{c}	288.7 ± 8.9 ^{f}	62.5 ± 13.7 ^{b}	144.6 ± 2.6 ^{d}	197.6 ± 2.3 ^{e}	36.4 ± 0.7 ^{a}
2-Furoic acid	55.5 ± 5.3 ^{b}	60.7 ± 1.8 ^{b}	9.4 ± 2.1 ^{a}	99.2 ± 3.3 ^{c}	95.1 ± 1.0 ^{c}	n.d.
d. Alcohols						
2,3-Butanediol	1127.8 ± 43.7 ^{e}	795.0 ± 44.3 ^{d}	372.5 ± 3.3 ^{b}	506.1 ± 15.5 ^{c}	1209.7 ± 38.5 ^{e}	285.9 ± 4.4 ^{a}
Benzyl alcohol	45.6 ± 4.6 ^{b}	41.5 ± 1.0 ^{ab}	38.5 ± 4.5 ^{ab}	38.3 ± 2.9 ^{ab}	42.0 ± 4.5 ^{ab}	32.9 ± 4.6 ^{a}
Phenylethyl alcohol	143.0 ± 9.1 ^{bc}	156.4 ± 3.8 ^{c}	109.3 ± 4.5 ^{a}	129.5 ± 2.4 ^{b}	190.0 ± 8.9 ^{d}	106.6 ± 5.7 ^{a}
Benzenacetaldehyde	21.3 ± 9.7 ^{d}	23.7 ± 5.0 ^{d}	12.4 ± 0.4 ^{b}	15.7 ± 2.1 ^{c}	22.1 ± 5.0 ^{d}	8.7 ± 1.2 ^{a}

e. Terpenes						
Myrcene	11.4 ± 0.4 ^{c}	11.4 ± 0.4 ^{c}	9.0 ± 0.3 ^{a}	9.8 ± 0.4 ^{ab}	16.5 ± 0.3 ^{d}	10.2 ± 0.3 ^{b}
Limonene	47.3 ± 1.1 ^{bc}	51.4 ± 2.8 ^{c}	46.2 ± 1.8 ^{bc}	46.2 ± 3.5 ^{bc}	19.2 ± 0.4 ^{a}	44.8 ± 2.5 ^{b}
ß-Trans-ocimene	16.6 ± 0.6 ^{ab}	17.4 ± 1.4 ^{b}	14.0 ± 1.1 ^{ab}	16.1 ± 1.1 ^{a}	24.4 ± 0.5 ^{c}	14.5 ± 1.5 ^{ab}
f. Acids, Esters, Ketones, Pyrans, Pyrroles						
Acetic acid	11130.2 ± 650.7 ^{e}	10367.7 ± 138.6 ^{de}	3826.5 ± 178.4 ^{b}	8684.5 ± 233.1 ^{c}	10239.6 ± 134.1 ^{d}	2823.0 ± 129.3 ^{a}
2-Heptanone	9.7 ± 0.5 ^{b}	9.0 ± 0.5 ^{ab}	7.9 ± 0.3 ^{a}	7.8 ± 0.2 ^{a}	16.3 ± 0.5 ^{c}	10.2 ± 0.6 ^{b}
2-Nonanone	26.0 ± 1.6 ^{ab}	27.8 ± 0.5 ^{abc}	27.4 ± 6.5 ^{ab}	22.9 ± 1.2 ^{a}	35.6 ± 0.9 ^{c}	33.9 ± 2.4 ^{bc}
2-Acetylpyrrole	71.9 ± 7.7 ^{c}	68.7 ± 3.8 ^{c}	32.2 ± 2.4 ^{b}	74.8 ± 1.0 ^{c}	72.2 ± 2.0 ^{c}	21.9 ± 1.4 ^{a}
Isoamyl acetate	16.3 ± 0.6 ^{bc}	14.5 ± 0.2 ^{ab}	12.6 ± 0.7 ^{a}	12.8 ± 0.9 ^{a}	38.8 ± 2.6 ^{d}	18.2 ± 1.2 ^{c}
2,3-Dihydro-3,5-dihydroxy-6-Methyl-4 H-pyran-4- one (DDMP)	437.7 ± 82.2 ^{c}	904.6 ± 210.4 ^{d}	90.9 ± 10.3 ^{a}	259.5 ± 9.7 ^{b}	1798.3 ± 177.2 ^{e}	n.d.

Mean values ± standard deviations

Different superscripts per component indicate significant differences ($p < 0.05$) among samples

n.d. : not detected

7.5 CONCLUSIONS

Substitution of sucrose in dark chocolate with palm sap sugar has potential for the development of dark chocolate products with a distinctive flavour/aroma. Moreover, palm sap sugar-sweetened chocolate, as a healthier chocolate, does not only contain additional minerals, vitamins, anti-oxidative and anti-carcinogenic compounds compared to standard chocolate, but may also be beneficial for diabetics. The aroma profile of palm sap sugar sweetened chocolate was marked with high concentrations of pyrazine based compounds and also with the presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)- pyran-4-one (DDMP) which was not found in sucrose sweetened dark chocolate.

Low particle density and high moisture content of the palm sap sugars in combination with the presence of reducing sugar were the main reasons for the lighter colour, higher hardness and higher viscosity of the resulting chocolates sweetened with palm sap sugar . With further improvements in processing technology, palm sap sugar can serve as a better and healthier alternative to traditional sucrose resulting in chocolates with improved health benefits for health-conscious consumers and the entire consuming populace.

CHAPTER 7

INVESTIGATING THE RHEOLOGICAL, MICROSTRUCTURAL AND TEXTURAL PROPERTIES OF CHOCOLATES SWEETENED WITH PALM SAP SUGAR BY PARTIAL REPLACEMENT

This chapter is redrafted from :
Saputro, A.D., Van de Walle, D., Kadivar, S., Sintang, M.D.B., Van der Meeren, P., Dewettinck, K., (2017). Investigating the rheological, microstructural and textural properties of chocolates sweetened with palm sap-based sugar by partial replacement. European Food Research and Technology. DOI 10.1007/s00217-017-2877-3.

CHAPTER 7
INVESTIGATING THE RHEOLOGICAL, MICROSTRUCTURAL AND TEXTURAL PROPERTIES OF CHOCOLATES SWEETENED WITH PALM SAP SUGAR BY PARTIAL REPLACEMENT

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7.1 ABSTRACT

Due to its physicochemical characteristics, utilisation of palm sugar as chocolate sweetener results in different quality attributes of chocolate (Chapter 5 and 6). In this work, a thorough investigation about the influence of palm sugar on the rheological, microstructural and textural characteristics of chocolate was carried out through partial replacement of sucrose as chocolate sweetener. Accordingly, 5 sucrose-palm sugar blends with different palm sugar (PS) proportion, namely PS0, PS25, PS50, PS75, and PS100 were used as chocolate sweetener. The results showed that the Casson yield value of chocolate containing palm sugar was lower than the Casson yield value of chocolate sweetened with pure sucrose which could be attributed to the presence of agglomerates in the chocolate suspension. However, palm sugar sweetened chocolate exhibited a higher Casson viscosity and thixotropy which could be mainly attributed to the presence of glucose and fructose and the relatively high moisture content. These factors also influenced the hardness of the chocolate to some extent. A lower melting temperature and enthalpy value of the sugar phase in chocolate were observed by DSC, whereas visualisation using SEM, polarised and normal light microscopy indicated increased agglomeration due to the presence of moisture, amorphous sugar and chemical "impurities". Rheological behaviour of molten chocolate, hardness, polarised and normal light microscopy were evaluated at a constant temperature of 40°C, 20°C, and 50^{o}C, respectively, while the melting profile was measured from 20^{o}C to 200^{o}C at a rate of 5^{o}C/min.

7.2 INTRODUCTION

Sugar properties such as particle size distribution, particle density, moisture content, and impurities, highly affect the rheological, textural and microstructural properties of chocolate. Chocolate with low particle size possesses a higher surface area, which increases the particle-particle interactions, resulting in a higher viscosity and hardness of chocolate (Afoakwa et al., 2008a; Afoakwa et al., 2008f). In terms of particle density, sugars with a lower particle density have a higher particle volume fraction, thus may create more particle-particle interactions in the chocolate suspensions (Shah et al., 2010; Sokmen and Gunes, 2006). The moisture content is also well-known to influence the viscosity of chocolate. Beckett (2008) stated that for every 0.3% extra moisture left in chocolate at the end of conching, an extra of 1% fat should be added to attain similar flow properties. The presence of moisture in chocolate can either dissolve sugar particles or create sticky patches on the surfaces of the sugar particles, creating particle agglomeration, which in turn affects particle-particle interactions. Aside from this, the utilisation of sugars containing glucose and fructose in chocolate production may also affect the properties of the chocolate produced. Due to their hygroscopicity, fructose and glucose may absorb moisture from the environment, hence sticking sugar particles together (Beckett, 2008).

Palm sap sugar is still traditionally produced (Phaichamnan et al., 2010a; Purnomo, 2007) which may result in unstandardised physicochemical characteristics. In previous studies where different types of palm sap sugars were utilised as chocolate sweetener (Chapter 5 and 6), it was reported that they clearly affected the quality attributes of dark chocolate (Saputro et al., 2016a; Saputro et al., 2016b). Therefore, to be able to get better insight about the impact of the chemical composition and the physical properties of palm sap sugar on the quality attributes of chocolate, partial replacement of sucrose with one type of palm sap sugar is required.

The aim of this work was to thoroughly investigate the impact of palm sugar on the rheological, microstructural and textural characteristics of dark chocolate. In this study, different sucrose-palm sugar blends were used as chocolate sweetener. The chocolates were produced in a small scale using an alternative processing method through a combination of mixing with a Stephan mixer and refining with a ball mill.

7.3 MATERIALS AND METHODS

7.3.1 Raw materials

Cocoa liquor (CM1 IC B), cocoa butter (pure prime pressed cocoa butter CB 1) and soy lecithin (SLS-578-12) were kindly provided by Belcolade (Erembodegem, Belgium). Coarse sucrose (Kristalsuiker EU2) was purchased from Tiense Suikerraffinaderij (Tienen, Belgium), while coarse palm sugar was purchased from Sari Nira Nusantara CV. (Yogyakarta, Indonesia).

7.3.2 Sample preparation

Dark chocolates with a total fat content of 36% were prepared according to the following formulation: 48.0% sugar, 34.4% cocoa mass, 17.2% cocoa butter and 0.4% lecithin. Coarse palm sugar (PS) and coarse sucrose (S) were blended in 5 different PS:S ratios; 100:0, 75:25, 50:50, 25:75, 0:100 (wt%) which were coded as PS100, PS75, PS50, PS25 and PS0, respectively. The specifications of the sugars can be found in Table 7.1.

Table 7.1. Specification of sugars used for chocolate productions

Sugar	Reducing sugar (%)		Protein (%)	Moisture content (%)	Particle Density (g/cm^3)
	Fructose	Glucose			
Sucrose	n.d*	n.d	n.d	0.22	1.57
Palm sugar	3.3	2.0	1.3	2.37	1.54

- n.d : not detected
- Sugar composition, moisture content, protein level and density were obtained through gas chromatography, Karl Fischer titration, Kjeldahl method and a pycnometer, respectively

The chocolates were produced using a combination of a Stephan mixer and a ball mill refiner, termed as an alternative processing, following the method described in Chapter 5 (Section 5.3.2.1). Afterwards, the chocolates were manually tempered and moulded which were conducted according the method described in Chapter 5 (Section 5.3.2.3).

7.3.3 Analytical methods

7.3.3.1 Rheological behaviour

Rheological behaviour of chocolate was measured according to the method described in Chapter 4 (Section 4.3.3).

7.3.3.2 Hardness

The hardness of the tempered chocolate bars was evaluated following the method described in Chapter 6 (Section 6.3.3.3).

7.3.3.3 Microstructural characteristics

The chocolate microstructure was observed using a Leica DM2500 microscope (Wetzlar, Germany) under polarised and normal light to observe sugar crystal arrangement and agglomerates in molten chocolate, respectively. For sugar crystal visualisation, a drop of molten chocolate which was previously heated at 55^{o}C for 1 hour was put on a glass slide. For the agglomerates visualisation, approximately 0.5 g of chocolate was diluted in 10 ml of isopropanol and heated in an oven at 50^{o}C for 1 hour. A cover slip was then placed over the sample and adjusted to ensure that the sample thickness was uniform. To stabilise the image of the samples during observation, the temperature of the glass slide support was set at 50^{o}C on a hot stage connected to a Linkam T95 System Controller (Linkam Scientific Instrument Ltd, Surrey, UK).

The surface morphology of both sugar and chocolate were visualised using a JSM-7100 F TTLS LV TFEG-SEM (Scanning Electron Microscopy) (Jeol Europe, Zaventem, Belgium) under high vacuum and at an accelerated voltage of 3 keV. Prior to electron beam targeting, the samples were vitrified in liquid nitrogen and transferred to a PP3000T device (Quorum Technologies, East Sussex, UK) at -140°C. Subsequently, the samples were allowed to sublime for 15 min at -70°C in order to remove frost, followed by sputtering a thin platinum film on the sample surface for 1 min.

7.3.3.4 Moisture Content

Moisture content of sugars was measured according to the method described in Chapter 3 (Section 3.3.2.1). Moisture content of chocolates was determined following the method described in Chapter 4 (Section 4.3.3).

7.3.3.5 Melting profile of sugar phase in chocolate

The melting profile of the sugar phase in chocolate was recorded with a Q1000 differential scanning calorimeter (DSC) equipped with a refrigerated cooling system (TA Instruments, New Castle, USA). Approximately 5 mg of tempered chocolate was hermetically sealed in aluminium cups. During thermal analysis, the samples were equilibrated at 20°C followed by a heating step to 200°C at a rate of 5°C/min as described by Saputro et al. (2016a).

7.3.3.6 Particle size distribution (PSD)

PSD of the chocolates was evaluated according the method described in Chapter 5 (Section 5.3.3.2).

PSD measurements were carried out following two sample preparation protocols, namely without and with sonication. The latter protocol was conducted by subjecting the chocolate solution in isopropanol to ultrasound waves at 80 Hz for 15 min. The sonication pre-treatment, was aimed to break the agglomeration. Therefore, the actual size of the particles can be obtained instead of the size of the agglomerates. A higher size reduction of the particles following sonication can be related to a higher degree of agglomeration (Saputro et al., 2016a).

7.3.4 Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). The chocolate properties were subjected to variance analysis (ANOVA) with 5% significance level. Testing for homogeneity of variances was performed with Levene Test. When the conditions for homogeneity of variances were fulfilled, the Tukey test was used to determine differences among samples. In case variances were not homogeneous, the Games–Howell test was performed. Principal component analysis (PCA) was used to visualise the interconnections among.

7.4 RESULTS AND DISCUSSION

7.4.1 Interconnections between moisture, sugar composition and particle size distribution in determining flow behaviour and hardness of chocolate

Flow behaviour and hardness of chocolate are very important quality attributes which should not be neglected. The former is very crucial, not only with regard to mouthfeel and consumer acceptance but also for the handling properties during application. The latter determines the snap which also influences consumer acceptance.

The results showed that the Casson yield value tended to decline as the proportion of palm sugar was increased. On the other hand, there was no clear trend on the Casson viscosity value as a function of palm sugar proportion in the chocolate. Nevertheless, it is worth to be mentioned that the highest Casson viscosity value was obtained from chocolate sweetened with PS100. Regarding the hardness, it significantly increased ($p < 0.05$) with the addition of palm sugar up to 50% and levelled off at higher palm sugar proportion. Both phenomena observed in the flow behaviour and hardness of chocolate were influenced by combination of moisture, sugar composition and particle size distribution (Afoakwa et al., 2008a; Afoakwa et al., 2008f; Beckett, 2008; Saputro et al., 2016a; Stortz and Marangoni, 2011). Aside from these, the lower particle density of palm sugar may also contribute to these phenomena to some extent (Shah et al., 2010; Sokmen and Gunes, 2006).

7.4.1.1 Flow behaviour

The Casson yield value, defined as a stress required to initiate the flow of molten chocolate (Afoakwa, 2010), is highly influenced by the surface of the particles. Mongia and Ziegler (2000) stated that at low stress, close to the yield value, particle-particle interactions, friction and adhesion dominate and, therefore, the yield value is proportional to the surface area of particles. It can be observed in Figure 7.1 that the chocolate sweetened with PS100 exhibited significantly lower ($p<0.05$) yield value than the other chocolates. Furthermore, it also can be seen that the Casson yield value increased as the proportion of palm sugar was decreased. This phenomenon can be explained by the fact that the chocolate containing palm sugar had a bigger particle size. Based on the PSD analysis, with sonication and without sonication pre-treatment methods, it was revealed that palm sugar sweetened chocolate showed a higher degree of agglomeration (Figure 7.2). Therefore, it seemed that the Casson yield value was more influenced by the surface area of the agglomerates than by the individual particles. Whereas the D(v,0.9) value is directly proportional to D(v,0.5), D(v,0.1) and Sauter mean D(3,2), it is inversely proportional to the specific surface area of the particles. Thus, chocolate sweetened with PS100, which had the highest degree of agglomeration D(v,0.9), exhibited the lowest agglomerate surface area, resulting in the lowest yield value. Apart from that, the fact that the actual size of the solid particles of palm sugar sweetened chocolates was slightly higher than that of sucrose sweetened chocolate (Figure 7.2), might also contribute to lower the Casson yield value. A similar observation was reported by Bolenz et al. (2006) when sucrose was partially replaced with lactose. In their study, the Casson yield value decreased, even though the Casson viscosity increased, as the proportion of lactose was increased. However, there was no further explanation for this phenomenon.

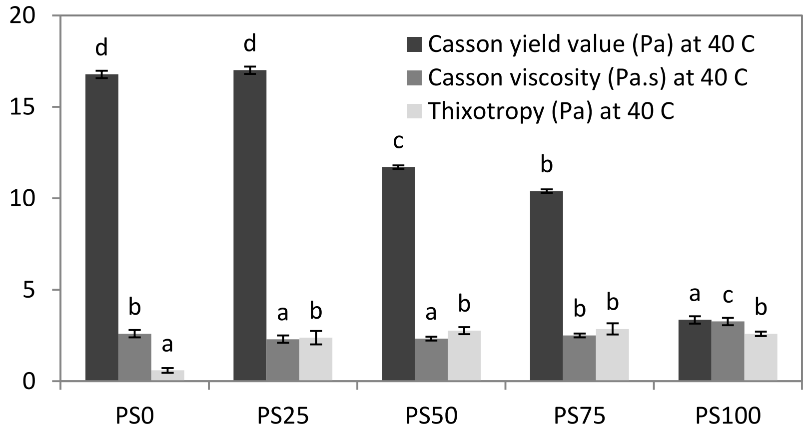

Figure 7.1. Flow behaviour of chocolates sweetened with sucrose-palm sugar blends

It can be seen in Figure 7.2 that chocolate with the highest moisture content (PS 100) exhibited the highest degree of agglomeration, followed by the ones sweetened with PS75, PS50 and PS25, consecutively. Contrary, almost no agglomeration was observed in chocolate sweetened with PS0. The agglomerates were formed due to the presence of moisture in the chocolate which may also induce the formation of amorphous sugar during chocolate production, causing the sugar particles to stick together and create agglomerates (Beckett, 2008). As can be seen in Table 7.1, moisture content of palm sugar (2.37%) was significantly higher than that of sucrose (0.22%). In addition, the presence of fructose and glucose, which are hygroscopic, in palm sugar may also contribute to the formation of agglomerates. It seemed that a slightly higher fat content in chocolates containing palm sap sugar, which was a result of moisture evaporation during chocolate processing, was not sufficient to reduce the particle agglomeration (Table 7.2). It can be seen in Table 7.2 that the higher the proportion of palm sugar in chocolate, the higher the moisture evaporation was, resulting in a higher fat content after processing.

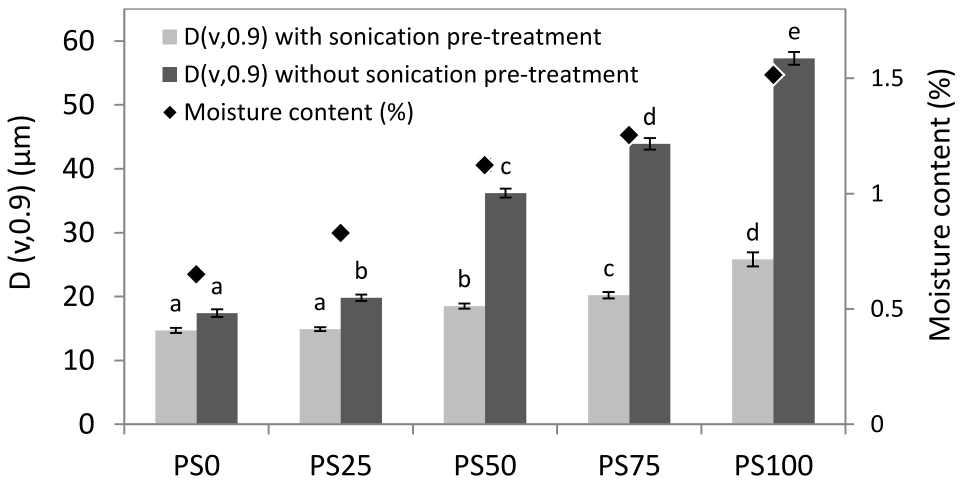

Figure 7.2. Particle size D(v,0.9) of chocolates sweetened with sucrose-palm sugar blends measured with and without sonication pre-treatment

Table 7.2. Moisture content of ingredients, moisture content of chocolates, evaporated moisture during processing and final fat content of chocolates after moisture evaporation

Chocolate	Moisture Content (%)			Final fat content of chocolate after moisture evaporation during processing (% wmb)
	Total Moisture of ingredients (%)	Moisture content of chocolate (%)	Evaporated moisture (%)	
PS100	1.68 ± 0.00^e	1.51 ± 0.00^e	0.17 ± 0.00^c	36.06 ± 0.00^c
PS75	1.40 ± 0.01^d	1.25 ± 0.00^d	0.15 ± 0.00^c	36.05 ± 0.00^c
PS50	1.18 ± 0.00^c	1.12 ± 0.01^c	0.06 ± 0.01^b	36.01 ± 0.01^b
PS25	0.92 ± 0.01^b	0.83 ± 0.00^b	0.09 ± 0.02^b	36.03 ± 0.01^b
PS0	0.65 ± 0.01^a	0.64 ± 0.01^a	0.01 ± 0.00^a	36.00 ± 0.00^a

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples
wmb: wet matter basis

The use of palm sugar in chocolate also influenced the Casson viscosity which refers to the internal friction during the flow. With the same amount of fat and dispersing agent, the chocolate viscosity is influenced by the combination of the particle volume fraction (Do et al., 2007; Saputro et al., 2016a; Shah et al., 2010; Sokmen and Gunes, 2006), particle size distribution (Afoakwa et al., 2008a; Mongia and Ziegler, 2000) and moisture content (Afoakwa, 2010; Beckett, 2008). It can be observed in Figure 7.1 that chocolate sweetened with PS100 exhibited the highest Casson viscosity, followed by chocolate formulated with PS75 and PS50, consecutively. However, chocolate formulated with PS0 exhibited a significantly higher ($p < 0.05$) Casson viscosity than chocolate sweetened with PS25 and PS50. This observation can be explained by the fact that even though chocolate sweetened with PS100 had the biggest D(v,0.9), resulting in a lower surface area (Figure 7.2), it also contained the highest moisture. It is well known that chocolates containing a higher amount of moisture require a higher amount of fat to attain similar viscosity to the ones with lower moisture. Moreover, the presence of fructose and glucose (reducing sugar) as hygroscopic material might also affect the viscosity. In addition, a higher particle size of chocolate sweetened with PS100 was due to a higher degree of agglomeration, which could be fragmented during shearing, creating a smaller particles with "wet" surfaces, resulting in a higher viscosity. Apart from that, a lower particle density of the palm sugar might also increase the Casson viscosity to some extent. Sokmen and Gunes (2006), reported that chocolate containing isomalt with particle density of 1.50 g/cm^3 exhibited a higher plastic viscosity, due to a higher solid volume fraction, than chocolate containing sucrose, maltitol and xylitol which had particle density of 1.60, 1.63 and 1.52 g/cm^3, respectively. A similar observation was also reported by Shah et al. (2010). In their study, it was mentioned that chocolate containing inulin HPX and HP had a higher viscosity than the one containing inulin GR which may be attributed to its higher solid volume fraction because the density of inulin HPX (0.47 g/ cm^3) and HP (0.49 g/ cm^3) were slightly lower than that of inulin GR (0.58 g/ cm^3).

When the proportion of palm sugar was reduced, it seemed that the effects of packing fraction and particle size distribution were more pronounced than the effects of moisture and reducing sugar. The packing fraction in chocolate, which depends on some factors such as particle arrangement, particle shape, and particle size distribution (PSD), can be described as the fraction of the space in chocolate filled with solid particles. Do et al. (2007) stated that increasing the PSD width (polydispersity) for a given particle volume fraction will reduce the viscosity of a suspension. Thus, chocolate sweetened with PS50 (span : 2.9) and PS25 (span : 2.8), which exhibited a broader/wider PSD than chocolate sweetened with PS0 (span 2.7)

(Figure 7.3B), had a lower Casson viscosity. In addition to a bigger PSD (Figure 7.2), chocolate sweetened with PS50, and PS25 had also more available "free" fat, resulting in a lower Casson viscosity than that of PS0 (Figure 7.1).

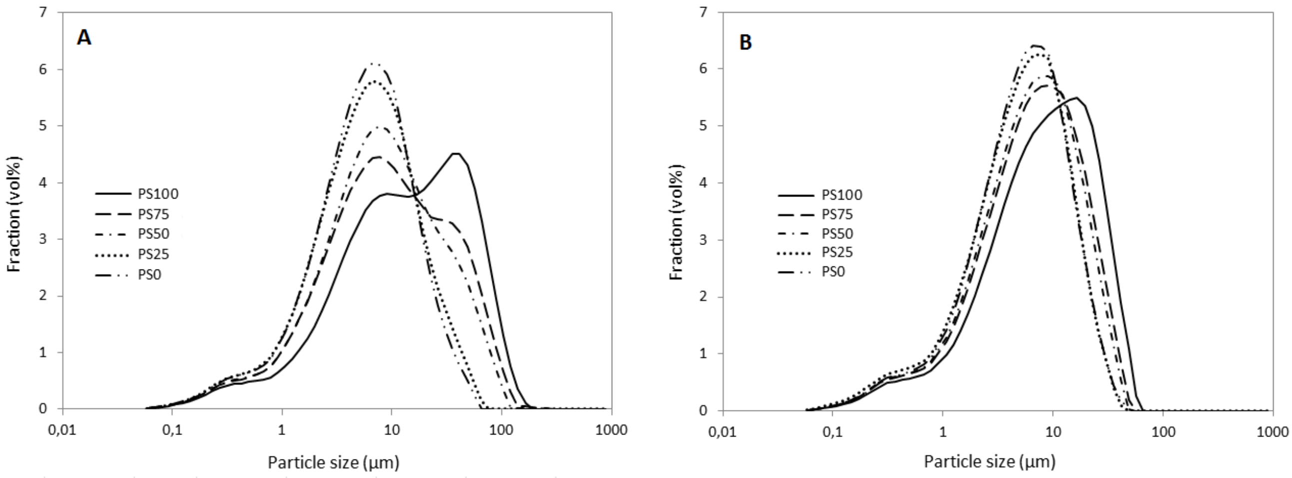

Figure 7.3. Particle size distribution curve of the chocolates sweetened with sucrose-palm sugar blends. (A) Measured without sonication pre-treatment, (B) Measured with sonication pre-treatment

Furthermore, the results showed that the bimodal PSD did not influence the chocolate viscosity. It can be seen in Figure 7.3A that chocolate sweetened with PS100, PS75, and PS50 exhibited a bimodal PSD, while chocolate sweetened with PS25 and PS0 exhibited a unimodal distribution. In the bimodal PSD, small particles may fill the voids between the big ones, reducing the amount of fat required to fill the voids (Do et al., 2007). Hereby, the presence of more available fat reduces the resistance to flow. However, in this case, the presence of a bimodal PSD did not considerably reduce the Casson viscosity. This phenomenon may be explained by the fact that the bimodal distribution observed in chocolate sweetened with PS100, PS75, and PS50 was formed due to the presence of agglomerates. During movement (shearing), agglomerates might be fragmented, thus the "wet" specific surface area increased, resulting in a higher amount of fat needed to coat the particles. This phenomenon reduced the "free" fat availability, thus increased the Casson viscosity.

The presence of agglomerates in the chocolate suspension can be confirmed by the thixotropy value of the chocolates (Do et al., 2007). It was stated that well-conched chocolates should not exhibit thixotropic behaviour (Servais et al., 2004). As seen in Figure 7.1, chocolate sweetened with sucrose (PS0) exhibited a lower thixotropy value ($p < 0.05$) than chocolates containing palm sugar, indicating less agglomeration in the chocolate. Aside from this, the presence of agglomeration in the chocolate was further confirmed by measuring the melting point and peak area of the sugar phase in chocolate. It was revealed that chocolate containing more moisture had a lower melting point and a narrower peak area than chocolate containing less moisture (Figure 7.4). The moisture content, which acts as a plasticiser, contributed in lowering the melting temperature of the sugar phase in chocolate. Additionally, the presence of chemical "impurities", such as fructose, glucose, proteins and minerals in palm sugar also played a role in lowering the melting point and enthalpy of the sugar phase in chocolate. Beckett et al. (2006) stated that the reduction of the melting point of a substance can also be correlated to the presence of impurities. In terms of enthalpy value, which corresponds to the peak area of the melting curve, chocolate sweetened with PS0 exhibited the highest enthalpy followed by chocolate sweetened with PS25, PS50, PS75, and PS100, consecutively.

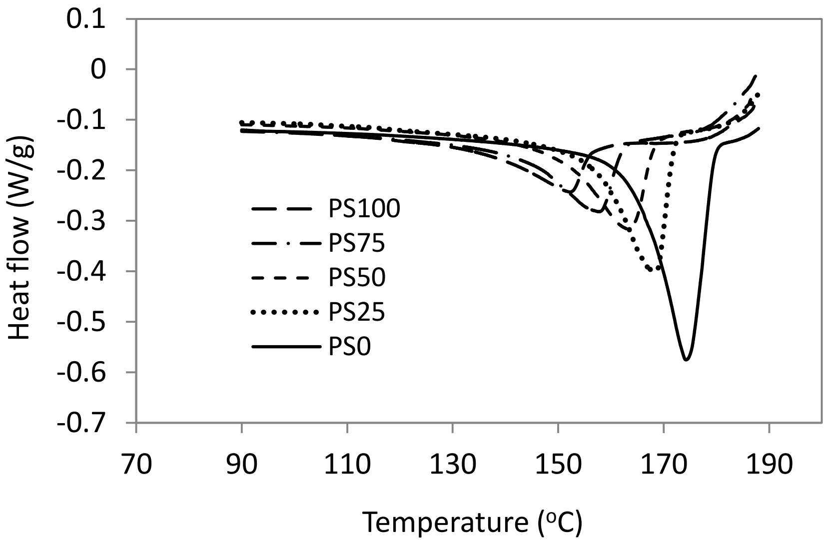

Figure 7.4. Melting profile of sugar phase in chocolate sweetened with sucrose-palm sugar blends

7.4.1.2 Hardness

The results showed that the hardness of the chocolates was highly influenced by the proportion of palm sugar (Figure 7.5). The increased moisture content was responsible for the increase of the hardness of chocolate sweetened with PS0 to PS50. Theoretically, it has been mentioned that chocolate with a smaller particle size results in a higher hardness due to more particle-particle interactions (Afoakwa et al., 2008e, f; Do et al., 2007). Therefore, chocolate sweetened with PS0 was expected to be the hardest chocolate among the samples in this study. However, in fact, chocolate sweetened with PS0 exhibited the lowest hardness. In this case, it seemed that the effect of moisture content was more pronounced than the effects of particle size. The higher the proportion of the palm sugar, the higher the moisture content of the chocolate was and consequently the higher the hardness of the chocolate became. Furthermore, the presence of fructose and glucose as hygroscopic material induced more amorphous sugar formation during chocolate production, which may result in a stronger sugar network (Beckett, 2009; Stortz and Marangoni, 2011). In addition, as previously discussed, since the particle density of palm sugar was lower than sucrose (Table 7.1), the particle volume fraction of the chocolate containing palm sugar become higher than that of chocolate containing sucrose (Shah et al., 2010; Sokmen and Gunes, 2006). In this case, more particle interaction could be formed, resulting in a harder chocolate, unless the solid particles of palm sugar are altered during milling process.

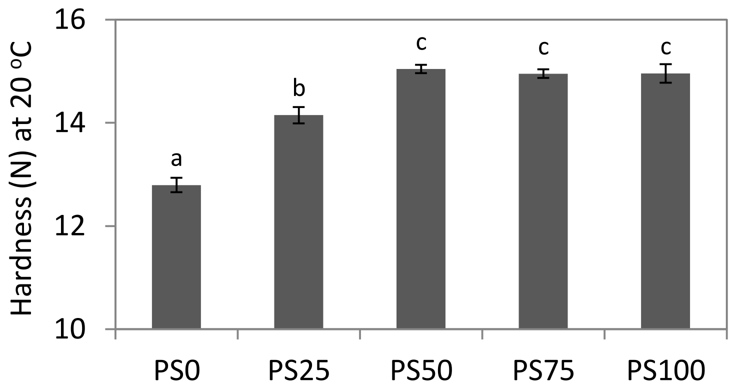

Figure 7.5. Hardness of chocolates sweetened with sucrose-palm sugar blends

At a palm sugar proportion higher than 50%, further addition of palm sugar did not increase the hardness of chocolates. It seemed that in the range of PS50 to PS100, the role of moisture and reducing sugar in creating sugar network decreased. The presence of a bimodal PSD, where the small particles may fill the gaps between the big ones, was suggested to be responsible for the similar hardness of chocolates sweetened with PS50, PS75, and PS100. In this case, the amount of fat required to fill the voids was reduced, thus more "free" fat were available. This condition might reduce the interactions among groups of particles and/or agglomerates, lowering the force required to penetrate the chocolate.

7.4.2 Microstructural properties

To further explain the rheological and textural characteristics of chocolate, microscopic investigation was performed. The results showed a clear variation in microstructure of sugars and chocolates.

7.4.2.1 Microstructural properties of sugar

The surface morphology of palm sugar and sucrose can be seen in Figure 7.6. The surfaces of palm sugar crystals, in contrast to sucrose, were covered with layers that cause the sugar crystals to stick to each other. The presence of these layers may be due to the relatively high moisture content, as well as due to the presence of fructose, glucose and amorphous parts.

7.4.2.2 Microstructural properties of chocolate

The surface morphology of chocolates sweetened with sucrose-palm sugar blends can be observed in Figure 7.7. From the images, it can be seen that all chocolates tended to have a similar surface morphology. This observation can be attributed to the use of a relatively high fat content (36%) in the formulation which optimally covered solid particles in the chocolates. The surface morphology of the chocolates was characterised by flaky surfaces, formed by crystallised cocoa butter as the suspending medium. This finding is in accordance with the work carried out by Afoakwa et al. (2009c) which reported that there were no clear differences in the chocolate crystal structure with different particle size (18-50 µm) at the same temper regime.

The presence of agglomerates in chocolates containing palm sugar can be clearly visualised in Figure 7.8. The chocolate sweetened with PS0 which contained the lowest amount of moisture and/or amorphous part exhibited well-dispersed particles (black colour) in isopropanol (grey colour), whereas the chocolates containing palm sugar exhibited particle agglomeration. The amount of agglomerates increased as the amount of palm sugar was increased. The images confirmed the importance of sonication pre-treatment prior to PSD measurement in order to be able to measure the actual size of the particles.

Polarised light microscopy was used to investigate the appearance of the sugar crystals and particle-particle interactions in the chocolates. Micrographs showed a clear variation in microstructure of the chocolates sweetened with different sucrose-palm sugar blends. In

Figure 7.9, the sugar crystals can be clearly seen in chocolate sweetened with PS0 which could be identified with a relatively big size of crystals with sharp/clear edges (whitish appearance). In contrast, similar sugar crystals could not be observed in chocolate sweetened with PS100. Instead, zooming in revealed unclear whitish edges overlapping, illustrating some possible connections between individual sugar particles and/or agglomerates, due to the presence of more amorphous part in the surface of palm sugar crystals. The fact that the chocolate sweetened with PS100 contained the highest amount of moisture and reducing sugar could induce more amorphous sugar formation during chocolate production. Moreover, it can be observed from Figure 7.9 that small particles filled the voids between big particles, which might increase packing fraction. Compared to the observation carried out by Afoakwa et al. (2009d), the sugar crystal network observed in this study was less dense. This can be due to the fact that the samples in this study contained a higher fat content (36%) than samples investigated by Afoakwa et al. (2009c).

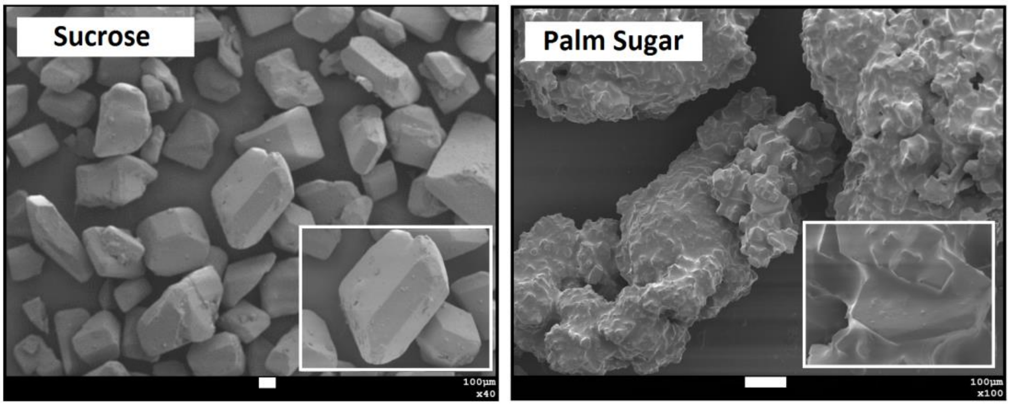

Figure 7.6. Morphology of sucrose and palm sugar crystals observed by scanning electron microscopy. The scale bar represents 100 µm.

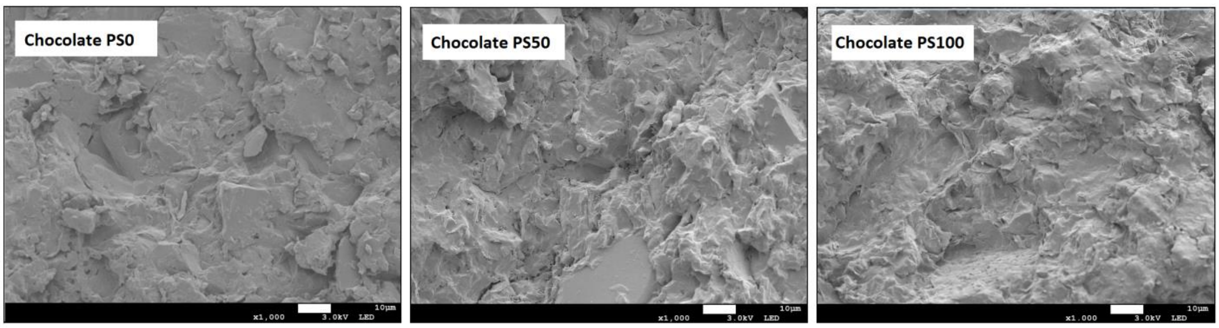

Figure 7.7. Morphology of chocolate sweetened with sucrose-palm sugar blends observed by Scanning Electron Microscopy. The scale bar represents 100 µm.

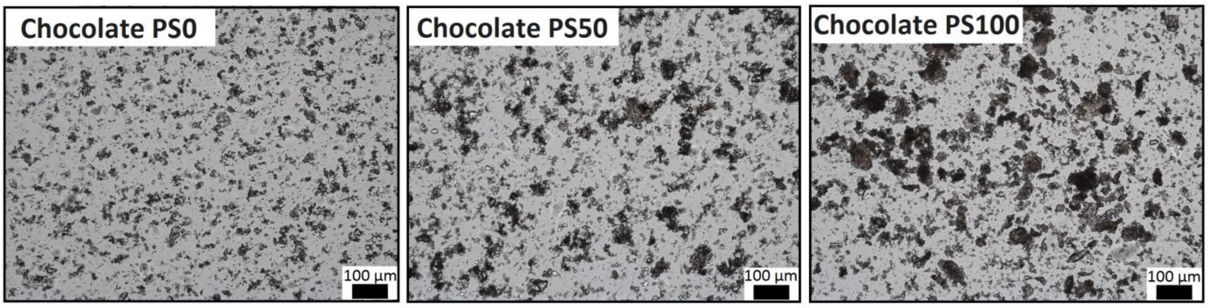

Figure 7.8. Particle agglomeration in isopropanol of chocolates sweetened with sucrose-palm sugar blend obtained with normal light microscopy. The scale bar represents 100 µm.

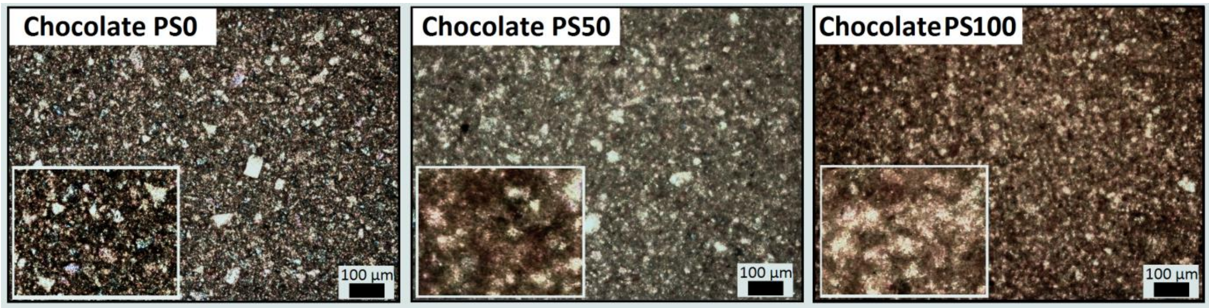

Figure 7.9. Micrographs of molten chocolate sweetened with sucrose-palm sugar blend obtained with polarised light microscopy. The scale bar represents 100 µm.

7.5 CONCLUSIONS

Partial replacement of sucrose with palm sugar as chocolate sweetener clearly influenced the rheological, microstructural and textural properties of palm sugar sweetened dark chocolate as a result of the presence of a higher moisture content and/or amorphous parts as well as the chemical “impurities” of palm sugar. The presence of moisture and/or amorphous parts created agglomeration of the solid particles, resulting in a lower yield value due to a lower surface area. On the other hand, the presence of a bimodal particle size distribution in palm sugar sweetened chocolate was due to the occurrence of agglomerates, which during shear might be fragmented, thus did not contribute in lowering the Casson viscosity. Moreover, due to the incidence of agglomerates, the thixotropy value of palm sugar sweetened chocolate was higher than that of sucrose sweetened chocolate. The existence of more moisture and/or amorphous sugar also increased the hardness of the chocolate to some extent which could be due to the sugar network formation.

The presence of more moisture and/or amorphous parts in palm sugar sweetened chocolate was investigated with thermal analysis which was supported by microstructural studies. The latter could visualise the impact of moisture and/or amorphous sugar as well as chemical "impurities" on the sugar and chocolate morphology, the agglomerates formation and the sugar network of molten chocolates.

CHAPTER 8

AROMA PROFILE AND APPEARANCE OF DARK CHOCOLATE FORMULATED WITH PALM SUGAR-SUCROSE BLENDS

This chapter is prepared for publication :
Saputro AD, Van de Walle D, Hinneh M, Van Durme J, Dewettinck K. Aroma profile and appearance of dark chocolate formulated with palm sugar-sucrose blends

CHAPTER 8
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8.1 ABSTRACT

Palm sap sugar is a natural alternative sweetener that can be made from the sap/nectar of several species of palm tree flowers. In this work, a thorough investigation about the impact of chemical composition and physical properties of palm sugar on the formation of aroma and appearance of dark chocolate was carried out. Five sucrose-palm sugar blends with different palm sugar (PS) proportion, namely PS0, PS25, PS50, PS75, and PS100 were used as sweetener. The results showed that a higher concentration of some alkyl pyrazines, alkyl furans, alkyl alcohols, 2-acetylpyrrole, and acetic acid observed in the palm sugar sweetened dark chocolate could be attributed to the aroma profile of palm sugar. In addition, there were some aroma volatiles which were only observed in palm sugar sweetened dark chocolate, such as 2-furoic acid, 3-hydroxy-2-butanone, 1-hydroxy-2-propanone, 2,3-dihydro-5-dihydroxy-6-methyl-4H-pyran-4-one, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP). The higher the proportion of palm sugar, the higher the concentration of these volatiles. However, the concentration of typical alkyl aldehydes in palm sugar sweetened dark chocolates was comparable to that in sucrose sweetened dark chocolate which might be associated with their minute amounts in palm sugar. The presence of more amorphous sugar was probably responsible for a higher concentration of aroma volatiles in chocolates containing palm sugar. Regarding the chocolate appearance, the effect of particle size in determining the colour was less pronounced than the effect of particle density and possible sugar network formation in the chocolate. The latter factor, might affect the surface roughness, resulting in different colour parameters. It seemed that the Maillard reaction during chocolate processing, which may influence the aroma profile and darkness of chocolate, occurred in much lesser extent that in the palm sugar production.

8.2 INTRODUCTION

Dark chocolate is a thick suspension, made from sugar and cocoa solids, dispersed in a continuous phase of cocoa butter (Afoakwa, 2016; Beckett, 2009). Its unique sensorial characteristics are influenced by not only the fineness, melting profile and flow behaviour, but also by its flavour (Chapter 5 and 6). The latter factor, which is defined by not only the taste but also aroma profile of chocolate, highly determines consumer preferences. In addition, the quality attribute appearance provides visual information about glossiness, roughness and colour (Afoakwa et al., 2008f) should not also be neglected in attracting consumers.

Aroma volatiles of chocolate, irrespective of the cacao variety/genotype, is highly influenced by postharvest handlings (fermentation, drying) and processing (roasting, conching) of cocoa material. Flavour precursors, namely free amino acids and peptides as well as reducing sugars, are formed during fermentation (Afoakwa, 2016; Aprotosoaie et al., 2016; Bonvehi, 2005; Kongor et al., 2016), while phenolic compounds are oxidised and polymerised, reducing bitterness and astringency of raw cocoa (Bonvehi, 2005; Kongor et al., 2016). Following fermentation, a significant increase of alcohols, organic acids, esters and aldehydes is also observed (Frauendorfer and Schieberle, 2008). During drying, major polyphenol-oxidising reactions catalysed by polyphenol oxidase take place, further reducing bitterness and astringency (Afoakwa et al., 2008b). During roasting, Maillard reactions and Strecker degradations occur consuming the flavour precursors formed during fermentation and producing desirable flavour compounds such as pyrazines, alcohols, esters, aldehydes, ketones, furans, pyrones and pyrroles. In this stage, low-boiling-point acids such as acetic acid are reduced (Afoakwa et al., 2008b; Counet et al., 2002b; Frauendorfer and Schieberle, 2008; Kongor et al., 2016). Residual volatile acids and moisture are evaporated during conching, which is the final stage of aroma development (Afoakwa, 2010). Aside from the aforementioned factors, the aroma volatiles of dark chocolate are also determined by the amount of cocoa butter (Afoakwa et al., 2009a), presence of amorphous sugar (Beckett, 2009; Saputro et al., 2016a; Saputro et al., 2016b), and particle size distribution of chocolate (Afoakwa et al., 2009a).

Appearance is the first attribute perceived by consumers to assess the quality of chocolate, and food in general, before making the decision to buy or not. In addition to glossiness, colour is one of the most common parameters used to evaluate the appearance of chocolate. Good quality of well-tempered dark chocolate can be seen from its glossy dark colour chocolate. The colour of well-tempered chocolate is highly influenced by particle-particle interaction of the solids in the chocolate suspension (Afoakwa et al., 2008e, f) and the level of moisture and reducing sugar (Saputro et al., 2016a).

In terms of taste, pure sucrose, as the most common chocolate sweetener, is considered as an inert ingredient contributing ‘‘only’’ to sweetness (Beckett, 1999). However, the use of alternative sugars can be an influential factor in determining the aroma profile of chocolate. An alternative sweetener that is capable of creating a distinctive not only chocolate flavour, but also appearance, due to its composition, is palm sap sugar (Saputro et al., 2016a; Saputro et al., 2016b). This unrefined sugar, which has various aroma volatiles and does contain not only sucrose but also protein and reducing sugars (Apriyantono et al., 2002; Phaichamnan et al., 2010a; Saputro et al., 2016a), is made from sap/nectar collected from the flower of palm tree species. It is widely used in soy sauce, cakes, desserts and many traditional foods in Southeast Asian countries (Purnomo, 2007). Apriyantono et al. (1996) reported that more than 70 volatiles, the majority of which derived from the Maillard reaction, such as furans, pyrazines and pyrroles, were identified in soya sauce sweetened with palm sugar.

In our previous studies (Saputro et al., 2016a; Saputro et al., 2016b) (Chapter 6) several types of palm sap sugar were used as chocolate sweetener. However, since palm sap sugar is still traditionally produced by applying different time and temperature regimes, it has various physicochemical characteristics. Due to this limitation, the influence of palm sugar on the chocolate aroma and appearance of chocolate could not be clearly defined. Therefore, in order to have a better understanding about the role of palm sap sugar composition and its physical properties in determining the formation of aroma profile and appearance of chocolate, a study on the partial replacement of palm sugar is needed. By doing partial replacement, identified aroma volatiles and the values of appearance parameters can be easily correlated to the physicochemical properties of palm sugar. The aim of this work was to thoroughly investigate the impact of palm sugar on the aroma profile and appearance of dark chocolate. In this study, the chocolates were produced in a small scale using an alternative processing method through a combination of mixing with a Stephan mixer and refining via a ball mill.

8.3 MATERIALS AND METHOD

8.3.1 Raw material

Cocoa mass (CM1 IC B), cocoa butter (pure prime pressed cocoa butter CB 1) and soy lecithin (SLS-578-12) were provided by Belcolade (Erembodegem, Belgium). Coarse sucrose (Kristalsuiker EU2) was obtained from Tiense Suikerraffinaderij (Tienen, Belgium), while coarse palm sugar was purchased from Sari Nira Nusantara CV. (Yogyakarta, Indonesia).

8.3.2 Sample Preparation

Dark chocolates were prepared according to this formulation: 48.0% of sugar, 34.4% of cocoa mass, 17.2% of cocoa butter and 0.4% of lecithin, with a total fat content of 36%. Coarse palm sugar (PS) was partially replaced with sucrose (S), which were manually blended in 5 different PS:S ratios; 100:0, 75:25, 50:50, 25:75, and 0:100 (wt%) coded as PS100, PS75, PS50, PS25 and PS0, respectively. Dark chocolates sweetened with PS100, PS75, PS50, PS25 and PS0 were coded as Choc PS100, Choc PS75, Choc PS50, Choc PS25, and Choc PS0, respectively. The specifications of the sugars can be found in Table 8.1.

Table 8.1. Specification of sugars used for chocolate productions

Sugar	Reducing sugar (%)		Protein (%)	Moisture content (%)	Particle Density (g/cm^3)
	Fructose	Glucose			
Sucrose	n.d.	n.d.	n.d.	0.2	1.57
Palm sugar	3.3	2.0	1.3	2.4	1.54

- n.d. : not detected
- Sugar composition, moisture content, protein level and density were obtained through gas chromatography, Karl Fischer titration, Kjeldahl method and pycnometry, respectively

The chocolates were produced using a combination of a Stephan mixer and a ball mill refiner, termed as an alternative processing method as described by Saputro et al. (Saputro et al., 2016b) in Chapter 5 (Section 5.3.2.1). This method consisted of three steps, namely mixing using a Stephan Universal Machine UMC 5 (Stephan food service equipment GmbH, Hameln, Germany), refining using a ball mill (Duyvis Wiener, Koogaan de Zaan, Netherlands) and liquefaction using the Stephan mixer. Afterwards, the molten chocolates were manually tempered and moulded which were conducted according the method described in Chapter 5 (Section 5.3.2.3).

8.3.3 Analytical Methods

8.3.3.1 Particle size distribution (PSD) measurement

The PSD of palm sugar and sucrose was measured following the method described in Chapter 3 (Section 3.3.2.9). PSD of chocolates was measured according the method described in Chapter 5 (Section 5.3.3.2).

8.3.3.2 Aroma volatiles analysis

The aroma profile of the chocolates was analysed using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS), following the method described in Chapter 5 (Section 5.3.3.5). Tentative identification of the volatile compounds in the headspace was performed using the Wiley 275 library. Kovats retention indices were determined to confirm the identified compounds, by injecting a series of n-alkane homologues.

8.3.3.3 Appearance measurement

The appearance of the tempered chocolate bars was measured using a colorimeter (Minolta Model CM-2500D Spectrophotometer, Tokyo, Japan) which was calibrated using a white reference standard. The SCE-mode (Specular light excluded) values were recorded and the colour parameters were expressed in L*a*b* colour space system with L* (luminance ranging from 0 (black) to 100 (white)), a* (green to red) and b* (blue to yellow). From these values, parameters within $L^*C^*h^o$ colour space were calculated according to following equations : $h^o = \arctan(b^*/a^*)$ and $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$ (Afoakwa et al., 2008f). L*, C*, and h^o represents the lightness of the sample, degree of saturation and hue luminance, respectively (Hutchings, 2011).

8.3.4 Data analysis

Data analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). Aroma volatiles concentration and colour parameters were subjected to variance analysis (ANOVA) at a 5% significance level. Testing for homogeneity of variances was performed with Levene Test. Tukey test was used to determine the differences among samples, if the conditions for homogeneity of variances were fulfilled. However, in case variances were not homogeneous, Games–Howell testing was performed. Principal component analysis (PCA) was used to visualise the relationships between chocolates and their volatile composition.

8.4 RESULTS AND DISCUSSION

8.4.1 Particle size distribution

PSD is considered to have a direct influence not only on the fineness and viscosity but also on the aroma profile and appearance of dark chocolate (Afoakwa et al., 2009a; Afoakwa et al., 2008f; Saputro et al., 2016a). In a dark chocolate suspension, PSD of the chocolate is determined by mixture of cocoa and sugar particles. Thus, in this study, since the same batch of cocoa mass was used and each processing step was performed at the same fat level and under the same conditions, the sugar particles is the only factor influencing PSD of the chocolate. Therefore, in order to gain better understanding on the role of palm sugar in determining the PSD of chocolate, PSD of the sugars and chocolates was measured.

It can be seen in Table 8.2 that even though D(v,0.1) and D(3,2) of palm sugar (PS100) and sucrose (PS0) were comparable, PS100 exhibited higher D(v,0.9), D(v,0.5) and D(4,3) value than PS0. This showed that PS100 had bigger particle size than PS0. In addition, Table 8.2 also shows that the span value of PS100 was significantly higher ($p<0.05$) than that of PS0, indicating a broader PSD. This phenomenon confirmed the fact that, in contrast to pure sucrose, palm sugar is still traditionally and manually produced by farmer without a proper grading and sieving process. As a consequence, a diverse size of the sugar particles are obtained.

Table 8.2. Particle size distribution of sucrose and palm sugar

Chocolates	Distribution Percentiles (µm)			Derived Diameter (µm)		Span (-)
	D (v,0.9)	D (v,0.5)	D (v,0.1)	D (4,3)	D (3,2)	
PS0	926 ± 14[a]	504 ± 10[a]	210 ± 8[a]	542 ± 10[a]	371 ± 26[a]	1.4 ± 0.02[a]
PS100	1170 ± 16[b]	568 ± 12[b]	200 ± 3[a]	633 ± 10[b]	367 ± 07[a]	1.7 ± 0.02[b]

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

Table 8.3 shows that Choc PS100 had a significantly higher D(v,0.9) ($p<0.05$) than Choc PS75, Choc PS50, Choc PS25, and Choc PS0, subsequently. This result was due to the fact that the particle size of palm sugar was higher than that of sucrose (Table 8.2). As expected, since all the chocolate ingredients were ground at the same time using the same protocol, irrespective of the PSD measurement method, the D(v,0.9) value of the chocolate increased as the proportion of the palm sugar was increased. In this study, D(v,0.9) was used to represent the size of the solid particles in chocolate because it has been reported to have a very good correlation with what people actually feel during chocolate consumption (Beckett, 2008; van der Vaart et al., 2013). Apart from D(v,0.9), it can also be seen in Table 8.3 that D(v,0.5), D(v,0.1), D(4,3), D(3,2) and span values increased as the proportion of the palm sugar was increase. This showed that PSD of palm sugar highly determined the PSD of chocolate.

Table 8.3. Particle size distribution of non-sonicated and sonicated chocolates

Chocolate	PSD (without sonication pre-treatment)					Span (-)
	Derived Diameter (µm)		Distribution Percentiles (µm)			
	D (v,0.9)	D (v,0.5)	D (v,0.1)	D (4,3)	D (3,2)	
Choc PS0	15.6 ± 0.8^{a}	5.2 ± 0.1^{a}	1.1 ± 0.0^{a}	7. 2 ± 0.3^{a}	2.1 ± 0.1^{a}	2.7 ± 0.1^{a}
Choc PS25	19.8 ± 0.5^{b}	5.5 ± 0.1^{b}	1.1 ± 0.0^{a}	8.5 ± 0.2^{b}	2.1 ± 0.1^{a}	2.8 ± 0.0^{a}
Choc PS50	36.2 ± 0.7^{c}	7.4 ± 0.2^{c}	1.3 ± 0.0^{b}	13.8 ± 0.3^{c}	2.5 ± 0.0^{b}	2.9 ± 0.0^{ab}
Choc PS75	43.9 ± 0.9^{d}	8.1 ± 0.1^{d}	1.3 ± 0.0^{b}	16.4 ± 0.5^{d}	2.5 ± 0.1^{b}	3.0 ± 0.1^{b}
Choc PS100	57.3 ± 0.9^{e}	13.0 ± 0.3^{e}	1.8 ± 0.0^{c}	22.6 ± 0.5^{e}	3.1 ± 0.1^{c}	3.1 ± 0.2^{b}

Chocolate	PSD (with sonication pre-treatment)					Span (-)
	Derived Diameter (µm)		Distribution Percentiles (µm)			
	D (v,0.9)	D (v,0.5)	D (v,0.1)	D (4,3)	D(3,2)	
Choc PS0	14.8 ± 0.4^{a}	5.1 ± 0.1^{a}	1.1 ± 0.0^{a}	6.8 ± 0.1^{a}	2.1 ± 0.0^{a}	2.8 ± 0.1^{a}
Choc PS25	14.7 ± 0.3^{a}	5.0 ± 0.2^{a}	1.0 ± 0.1^{a}	6.7 ± 0.2^{a}	2.0 ± 0.1^{a}	3.4 ± 0.1^{b}
Choc PS50	18.5 ± 0.4^{b}	5.9 ± 0.1^{b}	1.1 ± 0.0^{a}	8.1 ± 0.2^{b}	2.2 ± 0.1^{b}	4.7 ± 0.1^{c}
Choc PS75	20.2 ± 0.5^{c}	6.3 ± 0.2^{c}	1.2 ± 0.0^{b}	8.8 ± 0.2^{c}	2.3 ± 0.1^{b}	5.2 ± 0.1^{d}
Choc PS100	25.8 ± 1.1^{d}	8.0 ± 0.3^{d}	1.4 ± 0.2^{c}	11.2 ± 0.3^{d}	2.6 ± 0.2^{c}	5.3 ± 0.1^{e}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

Based on our previous study using the same chocolate samples (Saputro et al., 2017) (Chapter 7), palm sugar sweetened chocolate (Choc PS100) had the highest degree of agglomeration, followed by Choc PS75, Choc PS50, Choc PS25, and Choc PS0, consecutively (Table 8.3). Almost no agglomeration of particles was observed in the sucrose sweetened chocolate (Choc PS0). The degree of agglomeration, represented by the difference of D(v,0.9) values between samples measured with and without sonication pre-treatment, occurred due to the presence of relatively high moisture as well as fructose and glucose as hygroscopic materials in palm sugar (Table 8.1). The presence of excess moisture, then, can either dissolve sugar particles or create sticky patches on the surfaces of the sugar particles, creating particle agglomeration (Beckett, 2008). The presence of moisture and/or monosaccharides was confirmed by the DSC analysis showing a lower melting point and enthalpy of sugar phase in palm sugar sweetened chocolates (Saputro et al., 2017). Aside from this, microstructural observation visualised the presence of agglomerates in the chocolate containing palm sugar (Saputro et al., 2017).

8.4.2 Aroma profile

8.4.2.1 The role of palm sugar in determining the aroma profile of dark chocolates

To date, analytical studies have identified more than 600 volatiles in cocoa and chocolate, with pyrazines, aldehydes, esters, alcohols, and acids being reported as key volatiles (Afoakwa, 2016; Aprotosoaie et al., 2016; Counet et al., 2002b). These volatile compounds confer certain sensations during chocolate consumption, which can be perceived as positive or negative notes. For instance, the positive notes are the volatiles which have aromatic, fruity, flowery characters. The secondary flavours, such as honey, malt, toffee, caramel and raisins are also described to some extent as desirable. The negative notes are those which have beany, pungent, tobacco, phenolic, mouldy and hammy/smoky characters (Afoakwa, 2016; Urbanski, 1992; Viaene and Januszewska, 1999). The volatiles which are responsible for aforementioned notes can be seen in Chapter 5 (Section 5.4.4).

In this study, around 40 volatiles consisting of mainly pyrazines, aldehydes, furans, alcohols, terpenes, esters and acids compounds were recorded. To confirm the identified volatiles, Kovats indices were also determined (Table 8.4). The data of the volatiles were then analysed using PCA to emphasise the variation and visualise the trend. PCA data analysis showed that (Figure 8.1) PCA explained more than 81% variance in the first 2 factors, with PC 1 : 60.0% and PC 2 : 21.2%.

It can be observed in Figure 8.1 that, as expected, the chocolates could not be clustered as they were located in 5 different positions in the PCA graph. Choc PS0 was characterised by high negative values in PC1 which exhibited high levels of 2-pentylfuran (musty, green note (Owusu et al., 2012)), limonene (fruity, citrus note (Piggoit and Paterson, 1994)), heptanal (green note (Owusu et al., 2012)), β-trans-ocimene (herbal, sweet note (Kadow et al., 2013)), 2-nonanone (floral, fruity note (Kadow et al., 2013; Zellner et al., 2008)), nonanal (soapy note (Tran et al., 2016)), isoamyl acetate (fruity, banana note (Ramos et al., 2014)) and 2-heptanone (fruity, floral, green note (Counet et al., 2002b; Owusu et al., 2012)). Regardless of the odour threshold, these aroma volatiles were typical cocoa beans volatiles, developed during fermentation and roasting process (Afoakwa et al., 2008b; Counet et al., 2002b; Frauendorfer and Schieberle, 2008; Kongor et al., 2016). Choc PS25 was characterised by high positive values in PC2 which exhibited high concentration of 3-methylbutanal (chocolate note (Afoakwa et al., 2008b, 2009a)), 2-methylbutanal (chocolate note (Afoakwa et al., 2008b, 2009a)), 2-heptanone (fruity, floral, green note (Counet et al., 2002b; Owusu et al., 2012), pentanal (pungent note (Aprotosoaie et al., 2016)), myrcene (spicy note (Kadow et al., 2013)), hexanal (green note (Tran et al., 2016)), and benzaldehyde (bitter, almond-like note (Tran et al., 2016)). Choc PS50 was characterised by negative values in PC2 exhibiting the presence of high concentration of benzyl alcohol which confer sweet and floral note (Rodriguez-Campos et al., 2012).

Choc PS75 was characterised by high positive values in PC1 which exhibited high concentration of 3-hydroxy-2-butanone (butter, cream note (Tran et al., 2016)), 2,5-dymethylpirazine (roasted, cooked note (Afoakwa et al., 2009a; Owusu et al., 2012)), 2,6-dymethylpirazine (roasted, cooked note (Afoakwa et al., 2009a; Owusu et al., 2012)), 2,3-butanediol (sweet, chocolate note (Ramos et al., 2014)), 2(3H)-dihydrofuranone (caramel, sweet note (Owusu et al., 2012)), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) (roasted, caramel note (Bonvehi, 2005; Krings et al., 2006)), 2-acetylpyrrole (nutty, popcorn-like note (Rodriguez-Campos et al., 2012; Tran et al., 2016), and acetic acid (sour, vinegar note (Counet et al., 2002a; Owusu et al., 2012). The fifth chocolate (Choc PS100) was characterised by high positive values in PC1, but also in PC 2 which exhibited high concentration of pentanal, hexanal, octanal, 2-pyrrolidinone, 2,3-dimethylpirazine, 2,3,5-trimetylpirazine, and 2,3,5,6-tetramethylpyrazine, and phenylethyl acetate (honey, floral note (Aprotosoaie et al., 2016)). Unlike Choc PS0, Choc PS25 and Choc PS50, the fourth (Choc PS75) and the fifth (Choc PS100) chocolates were not only characterised by high concentration of aldehydes, furans, terpenes and ketones, but also by pyrazines, alcohols and acids. This showed that, in general, more various aroma volatiles were observed in Choc PC75 and Choc PC100. It is also worth mentioning that there were only two volatiles which confer negative notes, namely pentanal (pungent note) and nonanal (soapy note). However, as these volatiles were present in all chocolates (Table 8.4), it can be said that in terms of aroma profile the addition of palm sugar as chocolate sweetener is highly likely acceptable to consumers.

As can be seen in detail, in Table 8.4, the chocolates containing palm sugar (Choc PS25, Choc PS50, Choc PS75, and Choc PS100) tended to have higher concentration of volatiles than the chocolates containing pure sucrose. The concentrations of the volatiles mostly had a propensity to increase as the proportion of palm sap sugar was increased. These volatiles were alkyl pyrazine, such as 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, methylpyrazine, 2,3,5-trimethylpyrazine, and 2-pyrrolidinone; alkyl furan, such as 2(3H)-dihydrofuranone and 2-furan methanol; alkyl alcohol, such as 2,3-butanediol and phenylethyl alcohol; 2-acetylpyrrole, and acetic acid. The presence of a higher level of amorphous phase in palm sap sugar-sweetened chocolate might be responsible for this phenomenon. As stated by Beckett (Beckett, 2008), if sugar is refined/milled together with cocoa mass, some of the aroma volatiles are absorbed by the amorphous sugar, thus, resulting in a more intense aroma volatiles of chocolate. Therefore, as shown in Table 8.3, chocolate containing palm sugar which had higher degree of agglomeration than that of chocolate sweetened with sucrose, had a higher concentration of volatiles (Table 8.4). As previously discussed in Chapter 5, chocolates sweetened with sugar containing higher glucose and fructose content as well as moisture induce more amorphous phase during chocolate production. Apart from this, the aroma volatiles of palm sugar itself also contributed to the increase of the aroma volatile concentration of chocolate. As reported by some researchers, palm sap sugar contain various aroma volatiles such as acids, aldehydes, pyrazines, furans, ketons, pyrans, alcohols, esters, and pyrroles based compounds (Apriyantono et al., 1996; Barlina, 2016; Ho et al., 2006b, 2007). Thus, with the presence of more amorphous phase, the more aroma volatiles of the palm sugar were trapped, creating a higher concentration of volatiles.

Table 8.4 also shows that several volatiles were only present in chocolate containing palm sugar, with their concentration tend to increase as the proportion of palm sugar was increased, from PS25 to PS100. This phenomenon supported the aforementioned fact that the aroma volatiles of palm sugar had a contribution to increase certain aroma volatiles of chocolate. It can be seen that some volatiles which were present in palm sugar such as 2-furoic acid, 1-hydroxy-2-propanone, 3-hydroxy-2-butanone (Apriyantono et al., 1996) 2,3-dihydro-5-dihydroxy-6-methyl-4H-pyran-4-one (DHM), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) (Ho et al., 2006b) were only present in chocolate containing palm sugar.

The presence of various aroma volatiles in palm sugar is caused by the occurrence of Maillard reaction during its processing (Apriyantono et al., 2002; Ho et al., 2008b; Purnomo, 2007). Reducing sugar (fructose and glucose) and protein, as Maillard reaction precursors, are found in the sap/nectar. The former precursor is naturally converted from sucrose by micro-organisms (Phaichamnan et al., 2010a; Vidanapathirana et al., 1983). During processing, hydrolysis reaction of sucrose to fructose and glucose will be accelerated (Ho et al., 2008b; Phaichamnan et al., 2010a). Therefore, more reducing sugars are available for the Maillard reaction. Among the volatiles generated in this reaction, the N-, O- and S-heterocyclic compounds have a desirable aroma volatiles (Wan Aida et al., 2008)

The high concentration of some types of volatiles in chocolates containing palm sugar may have also been influenced by Maillard reaction occurred during chocolate production. This reaction might take place because reducing sugars and protein are not only present in palm sugar but also in the cocoa mass and because heat is applied during chocolate production. Nevertheless, it seemed that the additional Maillard reaction may only occur in a lesser extent. The fact that the duration of the processing was rather short (2 hours) may have limited the chance for the possible occurrence of this reaction. Aside from this, during conching the temperature applied and/or the concentrations of free amino acids and sugars may have been too low for Maillard reactions (Hoskin and Dimick, 1983).

An interesting trend related to the concentration of limonene and 2-pentylfuran, grouped as lipophilic compounds (Roberts et al., 2003), as well as 2-nonanone was observed. In general, their concentration decreased as the palm sugar proportion was increased. It seemed that the chocolate matrix contributed to this observation to some extent. It is widely known that interactions among volatile compounds and food matrix rely on the physicochemical properties of the substances. With regard to this, in the chocolate matrix, cocoa butter can entrap lipophilic volatiles, resulting in a lower concentration of volatiles detected by the instrument. However, since all the chocolates in this study contain similar amount of fat, the decreased concentration of those volatiles might be related to the particle size of the chocolate (Afoakwa et al., 2009a). The increase in surface area along with decreasing particle size D(v,0.9) was suggested to facilitate volatiles release (Afoakwa et al., 2009a). It can be seen in Table 8.2 that irrespective of the PSD measurement method, the higher the proportion of palm sugar, the bigger the particle size D(v,0.9) observed, thus resulting in a lower volatiles release.

Another interesting trend was related to the concentration of aldehydes. In general, it can be seen that the concentration of some alkyl aldehydes, such as 2-methylbutanal, 3-methylbutanal, pentanal, hexanal, heptanal, octanal, nonanal, and benzaldehyde were relatively comparable in each type of chocolate. This phenomenon might be due to the low concentration of aldehydes present in palm sap sugars. In some studies, Ho et al., (2006b, 2007) reported that in palm sap sugar, aldehyde was present only in minute amount.

8.4.2.2 Odour activity value (OAV)

To be able to estimate the role of identified volatiles in determining the overall chocolate aroma, their odour activity values were calculated. This value is described as the ratio between concentration of a volatile compound and its odour threshold value (OTV) obtained from literature. The calculated odour activity values, then, can be used to estimate which volatile compounds can be detected by consumer during chocolate consumption. It is worth to be mentioned that, depending on its odour threshold, a volatile even in a high concentration may not always have a big contribution to the overall aroma of the chocolate.

Chocolate is a fat continuous dispersion, thus OTV of volatiles in oil as the medium were chosen for the references. Nevertheless, the food matrix also influences the volatile release to some extent (Afoakwa et al., 2009a), including the types of fat (Hyvönen et al., 2003). Hereby, with regard to the calculation of OAV, the matrix type of the references should be taken into account. It can be seen in Table 8.4 that the OTV references for almost half of the identified volatiles were not found. Thus, the aroma volatiles, which contributed to the overall chocolates aroma, could not be clearly defined. However, in this study, OAV of dark chocolates was still investigated in order to get some insights.

Table 8.4 shows that most of the aroma volatiles had OAV less than 1, meaning that those aroma volatiles might not be perceived during chocolate consumption. This may be explained by the fat matrix in the chocolate which is different from the ones mostly found in the literature *e.g.* sunflower oil, rapeseed oil, vegetable oil and olive oil (Gemert, 2011). From the limited number of volatiles with OAV higher than 1, it can be seen that acetic acid followed by 2-methylbutanal and 3-methylbutanal were some of the odourants which had contribution in defining the overall aroma of chocolate.

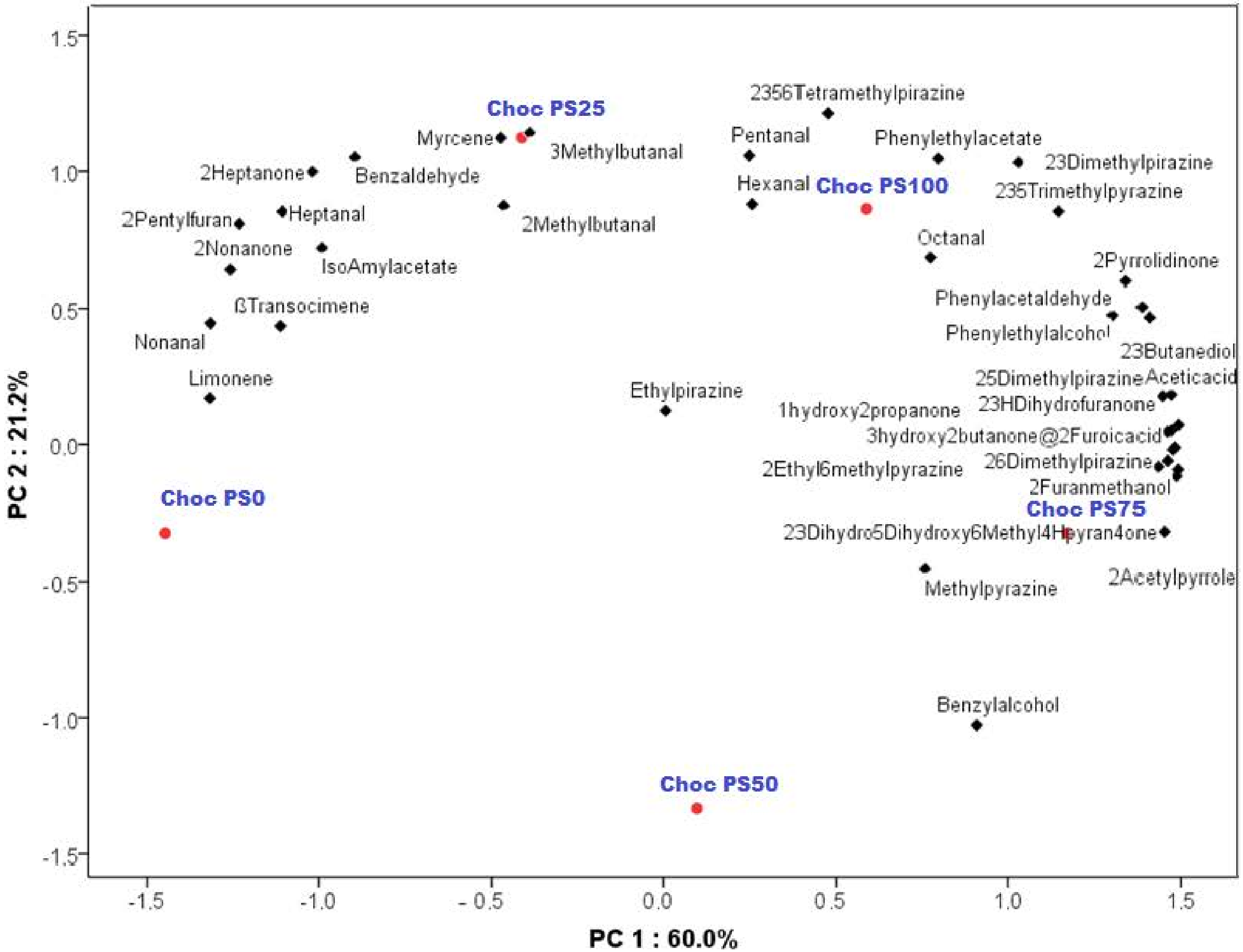

Figure 8.1. PCA (loadings and score plot) of aroma volatiles in chocolates sweetened with palm sugar - sucrose blends.

Table 8.4. Mean semi-quantitative concentrations of aroma volatiles (ng/g) in chocolates sweetened with palm sugar and sucrose blends identified by HS–SPME–GC–MS

Aroma Volatiles	Concentration (ng/g)					OTV (ng/g)	OAV					Kovat Index	
	Choc PS0	Choc PS25	Choc PS50	Choc PS75	Choc PS100		Choc PS0	Choc PS25	Choc PS50	Choc PS75	Choc PS100	Measu-rement	Literature
PYRAZINE													
Methylpyrazine	48.5 ± 4.4b	28.9 ± 5.7a	53.5 ± 4.0 b	56.1 ± 1.2 b	66.1 ± 3.3 c	27000 (b)	<1	<1	<1	<1	<1	1260	1286 (a)
2,5-Dimethylpyrazine	50.0 ± 3.3a	66.8 ± 1.7b	72.8 ± 4.2 b	80.3 ± 1.1 c	81.0 ± 0.2 c	2600 (a)	<1	<1	<1	<1	<1	1316	1346 (a)
2,6-Dimethylpyrazine	16.8 ± 1.2a	36.5 ± 2.1b	52.8 ± 7.6 c	67.3 ± 1.6 cd	62.8 ± 2.7 d	1021 (a)	<1	<1	<1	<1	<1	1323	1350 (a)
Ethylpyrazine	16.2 ± 2.5a	17.1 ± 2.5a	18.5 ± 1.1 a	15.1 ± 4.5 a	18.6 ± 0.1 a							1327	1354 (a)
2,3-Dimethylpyrazine	24.1 ± 2.0a	27.5 ± 0.6a	24.7 ± 3.8 a	27.9 ± 1.3 a	29.6 ± 1.9 a							1339	1365 (a)
2-Ethyl-6-methylpyrazine	8.9 ± 0.7a	10.8 ± 0.0b	12.6 ± 0.5 c	13.2 ± 0.3 c	13.2 ± 0.9 c	320 (a)	<1	<1	<1	<1	<1	1377	1381 (d)
2,3,5-Trimethylpyrazine	93.6 ± 3.8a	99.2 ± 2.4ab	94.0 ± 3.6 a	102.3 ± 3.2 b	101.0 ± 1.4 b	290 (ac)	<1	<1	<1	<1	<1	1395	1425 (a)
2,3,5,6-Tetramethylpyrazine	420.3 ± 14.0b	447.1 ± 16.6b	408.0 ± 6.1 b	440.5 ± 9.2 b	435.3 ± 24.2 b	38000 (b)	<1	<1	<1	<1	<1	1458	1495 (a)
2-Pyrrolidinone	11.2 ± 1.0a	30.5 ± 0.4b	26.7 ± 10.7 ab	38.6 ± 5.0 b	43.5 ± 7.5 b							1880	
ALDEHYDE													
2-Methyl butanal	24.9 ± 2.1bc	25.2 ± 1.5bc	22.7 ± 1.1 ab	18.9 ± 2.3 a	29.3 ± 0.8 c	2,2 - 152 (a)	<1 - 11	<1 - 11	<1 - 10	<1 - 9	<1 - 13	936	920 (a)
3-Methyl butanal	65.0 ± 6.2abc	69.1 ± 4.3bc	58.4 ± 4.6 ab	54.8 ± 6.6 a	75.0 ± 2.4 c	5,4 - 80 (a)	<1 - 12	<1 - 13	<1 - 11	<1 - 10	<1 - 14	937	924 (a)
Pentanal	43.9 ± 3.8ab	58.2 ± 3.9c	34.0 ± 3.3 a	54.3 ± 7.1 bc	45.6 ± 1.2 b	240 (a)	<1	<1	<1	<1	<1	977	988 (b)
Hexanal	69.2 ± 0.8b	89.4 ± 2.4c	57.7 + 5.5 a	85.7 ± 2.6 c	68.0 ± 2.7 b	75 - 300 (a)	<1	<1 - 1	<1	<1 - 1	<1	1075	1087 (a)
Heptanal	23.0 ± 2.6a	25.1 ± 2.1a	19.7 ± 0.4 a	18.7 ± 5.0 a	20.5 ± 2.0 a	250 - 500 (a)	<1	<1	<1	<1	<1	1181	1194 (a)
Octanal	61.6 ± 6.1a	78.5 ± 4.7ab	60.0 ± 2.0 a	83.0 ± 3.6 b	67.1 ± 1.4 ab	56 - 320 (a)	<1 - 1	<1 - 1	<1 - 1	<1 - 1	<1 - 1	1285	1298 (b)
Nonanal	71.2 ± 9.0 b	74.3 ± 8.2b	62.2 ± 7.3 ab	52.3 ± 5.3 a	58.5 ± 2.6 ab	1000 (b)	<1	<1	<1	<1	<1	1384	1407 (a)
Benzaldehyde	169.5 ± 9.6 b	163.5 ± 3.6 b	145.0 ± 9.4 ab	134.7 ± 2.2 a	172.3 ± 9.7 b	60 (a)	3	3	<1	<1	3	1519	1539 (a)
Phenylacetaldehyde	n.d	17.7 ± 1.8a	16.1 ± 2.1 a	19.8 ± 1.3 a	22.1 ± 5.0 a	22 (bc)	<1	<1	<1	<1	1>	1805	1660 (a)
FURAN													
2-Pentylfuran	35.8 ± 3.0b	35.8 ± 1.6b	30.7 ± 1.4 a	30.5 ± 1.2 a	32.5 ± 1.4 a	100 - 2000 (a)	<1	<1	<1	<1	<1	1228	1249 (a)
2(3H)-Dihydrofuranone	53.6 ± 1.4a	89.4 ± 1.8b	106.4 ± 21.9 bc	152.6 ± 3.6 d	131.9 ± 4.2 cd							1576	1649 (a)
2-Furan methanol	49.1 ± 1.6a	121.4 ± 2.3b	176.3 ± 10.7 c	248.9 ± 2.0 d	197.6 ± 2.3 d							1612	1655 (d)

2-Furoic acid	n.d	50.1 ± 3.7a	85.5 ± 6.7 b	106.6 ± 49.5 d	95.1 ± 1.0 c							1660	
Alcohol													
2,3-Butanediol	755.8 ± 10.7a	1053.1 ± 20.1bc	1030.7 ± 202.9 b	1258.1 ± 29.5 bc	1292.0 ± 48.1 c							1519	1557 (a)
Benzyl alcohol	40.0 ± 3.9a	41.8 ± 4.0a	49.2 ± 13.3 a	46.8 ± 4.6 a	42.0 ± 4.5 a							1771	1894 (a)
Phenylethyl alcohol	.4145.3 ± 7.4a	170.4 ± 4.3bc	167.8 ± 4.4 b	185.3 ± 2.9 cd	190.0 ± 8.9 d							1795	1929 (a)
TERPENE													
Myrcene	15.2 ± 0.5ab	16.2 ± 0.7b	14.6 ± 1.2 ab	13.7 ± 0.5 a	16.5 ± 0.3 b							1159	1179 (a)
Limonene	30.5 ± 1.8c	26.3 ± 1.7b	20.5 ± 0.8 a	21.4 ± 0.9 a	19.2 ± 0.4 a							1189	1202 (a)
β-Trans-ocimene	27.1 ± 2.2a	26.3 ± 1.7a	21.5 ± 2.2 a	24.4 ± 0.5 a	24.4 ± 0.5 a							1232	1253 (a)
ACID, ESTER, KETONE, PYRAN, PYRROLE													
Isoamyl acetate	38.1 ± 3.0b	36.7 ± 2.4b	31.3 ± 6.0 ab	22.5 ± 0.6 a	38.8 ± 2.6 b							1120	1117 (d)
2-Heptanone	16.5 ± 0.9bc	17.3 ± 0.8c	14.6 ± 1.3 ab	13.6 ± 0.3 a	16.3 ± 0.5 bc	300 -98000(a)	<1	<1	<1	<1	<1	1177	1192 (a)
3-hydroxy-2-butanone	n.d	17.6 ± 1.2a	20.9 ± 0.5 b	35.8 ± 1.2 d	24.1 ± 1.4 c							1278	
1-hydroxy-2-propanone	n.d	119.0 ± 6.1a	166.5 ± 0.7 b	215.6 ± 13.0 d	189.7 ± 4.6 c							1291	
2-Nonanone	47.5 ± 1.0c	44.0 ± 8.2bc	31.2 ± 3.2 a	33.4 ± 1.7 ab	35.6 ± 0.9 ab	100 (a)	<1	<1	<1	<1	<1	1381	1402 (a)
Acetic acid	3501.7 ± 19.8a	7577.7 ± 355.4b	8512.4 ± 493.7 c	10923.7 ± 142.2e	10239.6 ± 134.1 d	124 - 750 (a)	5 - 28	10 - 61	11 - 69	15 - 88	14 - 83	1430	1457 (b), 1439 (c)
Phenylethylacetate	45.5 ± 1.5a	46.9 ± 1.0a	44.2 ± 2.1 a	47.8 ± 2.4 a	47.7 ± 1.9 a							1726	1807 (a)
2,3-Dihydro-5-dihydroxy-6-methyl-4H-pyran-4-one (DHM)	n.d	22.6 ± 2.3 a	68.4 ± 18.5 b	124.7 ± 12.6 c	78.6 ± 3.0 b							1757	
2-Acetylpyrrole	32.7 ± 2.3a	50.4 ± 1.9b	66.7 ± 3.9 c	83.2 ± 0.9 d	72.2 ± 2.0 c							1835	1983 (a)
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one(DDMP)	n.d	618.2 ± 51.7ab	1223.7 ± 452.8bc	2163.7 ± 179.7d	1798.3 ± 177.2cd							2024	

Mean values ± standard deviations
Different superscripts per component indicate significant differences ($p < 0.05$) among samples
n.d. Not detected
Reference for odour threshold value (OTV) : a.Gemert (2011) b. Tran et al. (2015a) c. Frauendorfer and Schieberle (2008)
Reference for Kovat indices: a). Crafack et al. (2014) b). Muresan et al. (2000) c).Schnermann and Schieberle (1997) d). pherobase.com.

8.4.3 Appearance of dark chocolates

Table 8.5 shows that, in general, regardless of the proportion of palm sugar, chocolates containing palm sugar (Choc PS25, Choc PS50, Choc PS75, and Choc PS100) exhibited significantly higher L* value ($p<0.05$) than chocolate containing sucrose (Choc PS0), indicating a lighter chocolate. Furthermore, in detail, it can also be seen that L* value tended to increase as the proportion of palm sugar was increased. According to Afoakwa et.al (Afoakwa et al., 2008e, f) chocolate with small particles has a wider specific surface area, thus creating more particle-particle interaction. This condition is likely to result in a denser chocolate which can scatter more light, resulting in a lighter colour. As this was not the case with choc PS0, which had the smallest particle size D(v,0.9), it seemed that the chocolate colour was more strongly determined by the particle density of the palm sugar. A lower particle density of palm sugar which results in a higher particle volume fraction, may also increase the particle-particle interaction (Saputro et al., 2017; Shah et al., 2010; Sokmen and Gunes, 2006), resulting in a lighter colour (Saputro et al., 2016a). Apart from this, the fact that the chocolate which contains more moisture and reducing sugar induces more sugar network formation (Beckett, 2009; Stortz and Marangoni, 2011), therefore, may have also contributed to the increase of L* value. In our previous study (Chapter 6), the chocolate with a higher content of moisture, fructose and glucose had a higher hardness. The increased hardness might create different level of surface roughness to some extent, which then will scatter light distinctively, resulting in a different colour. Using digital vision system, Briones et al. (Briones et al., 2006) reported that chocolate with smooth surface had a higher L* value than that of chocolate with rough surface. This fact, may explain the phenomenon in which the Choc PS50 and Choc PS25 had a comparable L* value.

It can be seen in Table 5 that C and h^o value, which had a tendency to decrease as the proportion of palm sugar was increased, were inversely correlated to L* value. However, Afoakwa et al. (2008f) found that the L* value of the chocolates sweetened with sucrose of different particle sizes was directly correlated to C* and h^o value. The difference in this trend may be attributed to the four factors discussed above.

Table 8.5. Impact of partial replacement of palm sugar with sucrose on colour parameters of different chocolates

Colour parameters	PS0	PS25	PS50	PS75	PS100
L*a*b* colour space					
L*	24.12 ± 0.29^{a}	24.91 ± 0.45^{b}	24.83 ± 1.35^{b}	25.38 ± 0.22^{b}	25.98 ± 0.74^{c}
a*	9.29 ± 0.82^{c}	8.85 ± 0.16^{c}	8.53 ± 0.56^{bc}	7.55 ± 0.28^{a}	7.74 ± 0.36^{ab}
b*	9.07 ± 1.31^{b}	8.56 ± 0.30^{b}	8.10 ± 1.17^{ab}	6.72 ± 0.37^{a}	6.84 ± 0.74^{a}
L*c*h^{o} colour space					
L*	24.12 ± 0.29^{a}	24.91 ± 0.45^{b}	24.83 ± 1.35^{b}	25.38 ± 0.22^{b}	25.98 ± 0.74^{c}
c*	12.98 ± 1.51^{b}	12.31 ± 0.29^{b}	11.77 ± 1.21^{ab}	10.11 ± 0.44^{a}	10.33 ± 0.75^{a}
h^{o}	44.16 ± 1.64^{a}	44.02 ± 0.78^{a}	43.31 ± 2.34^{ab}	41.63 ± 0.80^{c}	41.32 ± 1.83^{c}

Mean values ± standard deviations from five times analysis.
Different superscripts per parameter indicate significant differences ($p < 0.05$) among samples

The L* value in L*a*b* colour space was directly proportional to the a* and b* values, which had a tendency to decrease as the proportion of palm sugar was increased. This observation confirmed the influence of palm sugar on the colour development of dark chocolate. In our previous study (Chapter 6), using different types of palm sap sugar, the impact of palm sugar on the a* and b* parameters was not completely clear.

As mentioned in the Chapter 5, during the production of palm sugar sweetened chocolate, additional Maillard reaction might only occur in a lesser extent than in the production of the palm sugar. Maillard reaction, which uses reducing sugar and protein as precursor, is well known to influence not only the aroma volatiles but also the darkness of food products. However, by increasing the proportion of palm sugar, the colour of chocolate did not become darker (Table 8.5), but, in contrast, the colour became lighter. Therefore, it may be more precise now to say that in terms of the colour of the chocolate, the role of the Millard reaction in the production of palm sugar sweetened chocolate appeared to be negligible.

8.5 CONCLUSIONS

Partial replacement of sucrose by palm sugar as chocolate sweetener underlined the role of palm sugar in determining the aroma profile and appearance of dark chocolate. By doing partial replacement, the role of aroma volatiles of palm sugar, the chemical composition of palm sugar, especially glucose, fructose and protein; the particle density and the moisture content of palm sugar could be well defined. Aroma profile analysis revealed that the aroma profile of palm sugar highly influenced the aroma profile of dark chocolate, which could be marked with a higher concentration of some alkyl pyrazines, alkyl furans, alkyl alcohols, 2-acetylpyrrole, and acetic acid observed in the palm sugar sweetened chocolate. In addition, some aroma volatiles present in palm sugar were only detected in palm sugar sweetened dark chocolate, such as 2-furoic acid, 3-hydroxy-2-butanone, 1-hydroxy-2-propanone, 2,3-dihydro-5-dihydroxy-6-methyl-4H-pyran-4-one, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP). An increase in the proportion of palm sugar tended to increase those volatiles concentration. Related to the influence of moisture and reducing sugar, it can be concluded that more amorphous sugar was responsible for a more intense aroma volatiles of chocolate. Nevertheless, due to a minute amount of alkyl aldehydes found in the palm sugar, their concentration in palm sugar sweetened dark chocolate were comparable to their concentration in sucrose sweetened dark chocolate.

The effect of particle size in determining the appearance of palm sugar sweetened dark chocolate, which is assessed by its colour, was less pronounced than the effect of particle density and possible sugar network formation in the chocolate. The latter factor might further influence the surface roughness, determining the colour. Even though reducing sugar and protein were present in the palm sugar, the role of the Maillard reaction during the chocolate production in determining the colour of the chocolate appeared to be negligible.

CHAPTER 9

FEASIBLE APPROACHES FOR SMALL-SCALE CHOCOLATE PRODUCERS TO IMPROVE FLOW BEHAVIOUR OF CHOCOLATE FORMULATED WITH PALM SAP SUGAR

Part of this chapter was presented in:
Saputro AD, Van de Walle D, González MP, Dewettinck K (2017). Controlling the flow behaviour of dark chocolate formulated with coconut sugar produced by combination of ball mill and liquefier device. 22nd National Symposium on Applied Biological Sciences, Belgium.

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9.1 ABSTRACT

The use of palm sap sugar as chocolate sweetener results in chocolate with relatively high viscosity due to the presence of high moisture and/or amorphous sugar. To overcome this problem, approaches to maintain the chocolate viscosity that can be easily and feasibly applied by small-scale chocolate producers are highly required. In this work, modification of processing method using a longer duration of the vacuum process and modification of formulation by increasing lecithin concentration were challenged. To evaluate the effect of vacuum process, palm sap sugar-sweetened chocolates were produced without vacuum process (CPS VP0), with 10 min of vacuum process (CPS VP10) and with 60 min of vacuum process (CPS VP60) during dry mixing. As reference, sucrose-sweetened chocolate was produced using 10 min of vacuum process. Ten minutes of vacuum process was the vacuum duration used during dry mixing in the previous studies. The results showed that the use of 60 min of vacuum process was most effective in evaporating moisture which resulted in a lower viscosity. The effectiveness of 60 min vacuum process in reducing the moisture was indicated with a higher melting temperature of sugar phase in chocolate and a lower degree of agglomeration analysed using PSD measurement, stress sweep and visualised using microscopy. Hence, 60 min of vacuum process was, then, selected for the study on production of chocolates with a higher lecithin concentrations. Palm sap sugar-sweetened chocolates produced with 60 min of vacuum process were formulated with 0.6%, 0.7% and 0.8% lecithin. As reference, palm sap sugar-sweetened chocolates with 0.4% lecithin was also produced. The result showed that the higher the lecithin concentration, the higher the Casson yield value and the lower the Casson plastic viscosity were observed. Thus, the amount of lecithin added into the chocolate should consider the application of chocolate.

9.2 INTRODUCTION

The use of palm sap sugar has been shown to highly influence the quality attributes of chocolates (Chapter 5-8). The “impurities” present in palm sap sugar such as moisture, reducing sugars, proteins and Maillard reaction products are responsible for the distinct chocolate characteristics. The presence of moisture and/or amorphous sugar induces the formation of particle agglomeration in chocolate, affecting not only grittiness but also other quality attributes of the chocolate, especially the rheological properties. As previously discussed in Chapter 1, 4, 5, 6 and 7, rheological behaviour of chocolate is very important because of its effect on the sensory characteristic and handling properties of the chocolate (Aidoo et al., 2013; Beckett, 2009; Mongia and Ziegler, 2000). Aside from this, other quality attributes of chocolate, more particularly hardness, can also be correlated to the rheological behaviour because their values are influenced by the same factors, namely particle size distribution, fat content, moisture content and particle density (Afoakwa et al., 2008e; Do et al., 2007; Saputro et al., 2016a; Sokmen and Gunes, 2006). Hence, in this work, the efforts to improve the quality of palm sap sugar-sweetened chocolate were performed by improving the rheological behaviour of chocolate.

Two approaches commonly used to maintain the rheological behaviour of chocolate are related to the formulation and processing method modification (Afoakwa, 2010; Beckett, 2009). The first approach can be done by adding more dispersing agent and cocoa butter as well as by changing the types of dispersing agent (Afoakwa, 2010; Beckett, 2009; Schantz and Rohm, 2005a). The second approach can be conducted by adjusting the conching duration and temperature in the conventional processing, as well as by adding other processing steps such as coarse conching process prior to refining and/or liquefaction process after refining in the alternative processing (Beckett, 2009; Bolenz et al., 2014b).

This work aimed at investigating the extent of lecithin concentration and/or application of vacuum process during dry mixing, as the most feasible methods applied by small-scale chocolate makers, that can reduce particle agglomeration and improve the flowability of palm sap sugar-sweetened chocolate. The outcome of this work is important for small-scale chocolate makers to decide on the duration of vacuum process and/or the percentage of lecithin needed to create chocolate with desired flow properties. Moisture content analysis, melting temperature of sugar phase in chocolate, rheological behaviour and microstructural visualisation were used to evaluate these two approaches.

9.3 MATERIALS AND METHODS

9.3.1 Raw materials

Cocoa liquor (CM1 IC B), cocoa butter (pure prime pressed cocoa butter CB 1) and soy lecithin (SLS-578-12) were kindly provided by Belcolade (Erembodegem, Belgium). Coarse sucrose (Kristalsuiker EU2) was purchased from Tiense Suikerraffinaderij (Tienen, Belgium), while coarse palm sugar was purchasd from Sari Nira Nusantara CV. (Yogyakarta, Indonesia).

9.3.2 Sample preparation

9.3.2.1 Vacuum treatment

Dark chocolates with a total fat content of 36% were prepared according to the following formulation: 48.0% sugar, 34.4% cocoa mass, 17.2% cocoa butter and 0.4% lecithin. Coarse palm sap sugar (CPS) was used as alternative sweetener, while coarse sucrose (CS) was used as reference. The specifications of the sugars can be seen in Table 9.1. In this work, chocolates were produced by a combination of a Stephan mixer and a ball mill refiner as described in Chapter 5 (Section 5.3.2.1 with slight modifications. The first type of chocolate was produced without application of a vacuum process (CPS VP0) and the second type of chocolate was produced with 10 min and 60 min of vacuum process during dry mixing stage (CPS VP10 and CPS VP60, respectively). As reference, sucrose-sweetened chocolate was produced using 10 min of vacuum process (CS Ref). Ten min of vacuum process was the vacuum duration applied during dry mixing in the previous studies (Chapter 4-8).

Table 9.1 Specification of sugars used for chocolate productions (dry matter basis)

Sweetener	Composition			Protein (%)	Moisture (%)	Density (g/cm^3)
	glucose (%)	fructose (%)	sucrose (%)			
Palm sap sugar	3.14	4.32	77.87	1.8	2.5	1.49
Sucrose	n.d.	n.d.	100	n.d.	0.3	1.57

Sugar composition, moisture content, protein level and density were obtained through gas chromatography, Karl Fischer titration, Kjeldahl method and a pycnometer, respectively
n.d. : not detected

9.3.2.2 Lecithin addition

Coarse palm sap sugar sweetened chocolates with 0.6%, 0.7% and 0.8% lecithin concentration labelled as CPS0.6%, CPS0.7%, and CPS0.8%, respectively, were also produced using a combination of Stephan mixer and ball mill refiner. As reference, palm sap sugar-sweetened chocolates with lecithin concentration of 0.4% (CPS0.4% Ref) was also produced. The chocolate production method was described in Chapter 5 (Section 5.3.2.1 using 60 min of vacuum process, instead of 10 min, during dry mixing. Regarding the lecithin addition, 0.2% lecithin was added at the beginning of wet mixing step, before milling process, while the remainder was added at the beginning of liquefaction step, after milling process. The formulation of the chocolates can be seen in Table 9.2.

Table 9.2 Formulation of dark chocolates with different lecithin concentrations

Ingredient	Mass (wt%)			
	CPS0.6%	CPS0.7%	CPS0.8%	CPS0.4% Ref
Sugar	48.00%	48.00%	48.00%	48.00%
Cocoa mass	34.42%	34.42%	34.42%	34.42%
Cocoa butter	16.98%	16.88%	16.78%	17.18%
Lecithin	0.60%	0.70%	0.80%	0.40%

9.3.3 Analytical methods

9.3.3.1 Moisture Content

Moisture content of chocolates was determined following the method described in Chapter 4 (Section 4.3.3).

9.3.3.2 Melting profile of sugar phase in chocolate

Melting profile of sugar phase in chocolate was measured according to the method described in Chapter 7 (Section 7.3.3.5).

9.3.3.3 Particle size distribution (PSD)

PSD of chocolates was measured following the method described in Chapter 5 (Section 5.3.3.2).

9.3.3.4 Rheological behaviour

Two types of rheological behaviour measurements were conducted, namely flow behaviour test (Chapter 4; section 4.3.3) and oscillatory stress sweep to determine the linear viscoelastic region (LVR). Oscillatory measurements were carried out at a frequency of 1 Hz. Prior to the measurement, a conditioning step at 40^{o}C for 10 min was needed. Afterwards, the chocolate sample was subjected to an increasing oscillatory stress from 0.01 Pa to 100 Pa. Graphs were obtained by plotting the complex modulus G* (Pa) as a function of oscillatory shear stress.

9.3.3.5 Microstructural properties

Microstructural properties of chocolates were visualised using polarised and normal light microscopy as described in Chapter 7 (Section 7.3.3.3).

9.3.4 Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). The chocolate properties were subjected to variance analysis (ANOVA) at 5% significance level. Testing for homogeneity of variances was performed with Levene Test. When the conditions for homogeneity of variances were fulfilled, the Tukey test was used to determine differences among samples. In case variances were not homogeneous, Games–Howell test was performed.

9.4 RESULTS AND DISCUSSION

9.4.1 APPLICATION OF VACUUM PROCESS

The application of vacuum during mixing/dry "conching-like" step did not significantly increase the amount of moisture evaporated in the production of chocolate sweetened with sucrose (Chapter 4). This phenomenon may be attributed to the fact that sucrose contains very low moisture (Table 9.1). Thus, it seems that the application of vacuum is more suitable for chocolate production with relatively high moisture ingredients, such as palm sap sugar.

9.4.1.1 Moisture content and melting profile of sugar phase in chocolate

Table 9.3 shows that palm sap sugar sweetened chocolate produced without application of vacuum process (CPS VP0) exhibited significantly higher moisture ($p<0.05$) than chocolate produced with the use of 60 min vacuum process (CPS VP60). Moreover, palm sap sugar sweetened chocolate produced with application of 10 min vacuum process (CPS VP10) had also significantly higher ($p<0.05$) moisture content than CPS VP60, but exhibited significantly lower ($p<0.05$) moisture content than CPS VP0. This phenomenon showed that the longer the vacuum duration, the lower the moisture content in the chocolate was observed. However, all the palm sap sugar-sweetened chocolates (CPS VP0, CPS VP10 and CPS VP60) still exhibited significantly higher moisture ($p<0.05$) than sucrose sweetened chocolate (CS Ref).

The amount of moisture and impurities present in chocolate can be linked to the melting temperature of sugar phase in chocolate. As can be seen in Table 9.3, CPS VP0 exhibited lower onset and melting peak temperature than CPS VP10 and CPS VP60. Moreover, regardless of the vacuum duration, palm sap sugar-sweetened chocolates had lower onset and melting peak temperature than sucrose-sweetened chocolate (CS Ref). This observation can be associated with a higher amount of moisture and impurities which acts as plasticiser, thus lowering the onset and melting temperature (Beckett et al., 2006; Ergun et al., 2010). In addition, it can also be seen in Table 9.3 that CPS VP0 had a lower enthalpy value than CPS VP10, CPS VP60 and CPS Ref, showing that CPS VP0 had a lower amount of sugar in crystalline state than CPS VP10, CPS VP60, and CS Ref. Sucrose sweetened chocolate which contained the lowest moisture and impurities had the highest onset, melting peak temperature and enthalpy value. In terms of width value, as previously discussed in Chapter 3 and 5**,** melting range of a substance relies on the presence of impurities. Substances with low impurities tend to have a narrow melting range. Therefore, as can be seen in Table 9.3, palm sap sugar sweetened chocolates had significantly higher ($p<0.05$) width values (15.78 - 18.69^{o}C) than CS Ref (7.4^{o}C).

Table 9.3 Moisture content and melting profile of sugar phase in dark chocolates produced with different vacuum duration

Chocolates	Moisture content (%)	Melting profile parameters			
		Onset (°C)	Peak Max (°C)	Width (°C)	Enthalpy (J/g)
CPS VP0	1.6 ± 0.1^{d}	135.3 ± 0.4^{a}	156.8 ± 0.5^{a}	17.8 ± 0.7^{c}	35.9 ± 2.9^{a}
CPS VP10	1.4 ± 0.0^{c}	139.6 ± 1.1^{b}	158.6 ± 0.4^{b}	15.8 ± 0.7^{b}	37.1 ± 2.5^{a}
CPS VP60	0.9 ± 0.0^{b}	145.4 ± 0.6^{c}	165.1 ± 0.7^{c}	18.7 ± 0.7^{c}	50.7 ± 1.7^{b}
CS Ref	0.4 ± 0.0^{a}	168.3 ± 0.4^{d}	177.3 ± 0.2^{d}	7.4 ± 0.1^{a}	77.2 ± 3.8^{c}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

9.4.1.2 Particle size distribution

As previously discussed, palm sap sugar which contains relatively high moisture and/or amorphous sugar induces particle agglomeration. The higher the moisture and/or amorphous sugar content, the higher the degree of agglomeration will be. The degree of agglomeration is demonstrated by the difference in D(v, 0.9) value between PSD measured with and without sonication pre-treatment. Hence, since CPS VP0 contained higher moisture than CPS VP10 and CPS VP60 (Table 9.3), the degree of agglomeration of CPS VP0 was higher than that of CPS VP10 and CPS VP60 (Table 9.4). On the other hand, due to a very low amount of moisture, almost no particle agglomeration was observed in sucrose sweetened dark chocolate.

Considering that the types of palm sap sugar used for chocolates production were the same, the difference of D(v,0.9) values among chocolates was most likely due to the moisture content of the mixture of chocolate ingredients before ball milling. The difference in moisture level likely influenced the breakage behaviour of the solid particles, mainly sugar, thus resulting in different particle sizes. CPS VP60 which had the lowest moisture content among palm sap sugar sweetened chocolates, exhibited the lowest D(v, 0.9) value, followed by CPS VP10 and CPS VP0 which had moisture content of 1.4% and 1.6%, respectively. The lower the moisture content, the more brittle the sugar particle were, and thus the smaller the size of the resulted particles. Furthermore, it can also be seen in Table 9.4 that D(v, 0.5), D(v, 0.1), D(4,3) and D(3,2) values of samples measured without sonication pre-treatment, as a function of vacuum duration, showed a similar trend to D(v, 0.9). However, D(v,0.9) of samples measured with sonication pre-treatment only exhibited a similar trend to D(v, 0.5) and D(4,3), but had a slightly different trend to D(v, 0.1) and D(3,2) which could be due to the inaccurate technique for very small particles. Regarding the span value, there were no clear trends observed in the samples measured using both methods. However, it is worth mentioning that the presence of agglomeration and breakage behaviour of particles during milling influenced the span value.

Table 9.4 Particle size distribution of chocolates produced with different vacuum duration measured with and without sonication pre-treatment

PSD (without sonication pre-treatment)

Chocolates	Distribution Percentiles (µm)			Derived Diameter (µm)		Span (-)
	D(v, 0.9)	D(v, 0.5)	D(v, 0.1)	D(4,3)	D(3,2)	
CPS VP0	71.8 ± 1.3^{cA}	26.4 ± 0.9^{dA}	3.4 ± 0.4^{cA}	32.6 ± 0.8^{dA}	4.6 ± 0.2^{cA}	2.6 ± 0.1^{aA}
CPS VP10	68.8 ± 1.7^{cA}	19.9 ± 0.8^{cA}	2.1 ± 0.1^{bA}	28.7 ± 0.8^{cA}	3.5 ± 0.2^{bA}	3,3 ± 0.1^{cA}
CPS VP60	26.2 ± 0.8^{bA}	7.4 ± 0.2^{bA}	1.4 ± 0.1^{aA}	11.1 ± 0.3^{bA}	2.7 ± 0.2^{aA}	3.4 ± 0.1^{cA}
CS Ref	20.2 ± 0.6^{aA}	6.2 ± 0.1^{aA}	1.5 ± 0.0^{aA}	9.0 ± 0.2^{aA}	2.7 ± 0.1^{aA}	3.0 ± 0.0^{bA}

PSD (with sonication pre-treatment)

Chocolates	Distribution Percentiles (µm)			Derived Diameter (µm)		Span (-)
	D(v, 0.9)	D(v, 0.5)	D(v, 0.1)	D(4,3)	D(3,2)	
CPS VP0	39.4 ± 0.8^{dB}	14.1 ± 1.3^{cB}	1.7 ± 0.1^{bB}	17.7 ± 0.6^{dB}	2.8 ± 0.0^{bB}	2.7 ± 0.3^{aA}
CPS VP10	31.4 ± 0.6^{cB}	8.6 ± 0.3^{bB}	1.1 ± 0.1^{aB}	12.9 ± 0.2^{cB}	2.2 ± 0.1^{aB}	3.5 ± 0.2^{bA}
CPS VP60	16.6 ± 0.6^{aB}	6.2 ± 0.2^{aB}	1.4 ± 0.2^{bA}	7.8 ± 0.2^{aB}	2.7 ± 0.2^{bA}	2.5 ± 0.2^{aB}
CS Ref	19.5 ± 0.4^{bA}	6.2 ± 0.2^{aA}	1.5 ± 0.1^{bA}	8.8 ± 0.2^{bA}	2.7 ± 0.2^{bA}	2.9 ± 0.1^{aA}

Mean values ± standard deviations from triplicate analysis.
Different superscripts (lowercase) in the same column indicate significant differences ($p < 0.05$) among samples, different superscripts (uppercase) in the same sample with different treatments (with and without sonication pre-treatment) indicate significant differences ($p < 0.05$) among samples

9.4.1.3 Rheological behaviour

Casson model is the most common model used to evaluate the rheological behaviour of chocolate (Chapter 1). However, in this experiment, in order to have more detailed information, oscillatory rheology measurement was also carried out. By conducting this method, linear viscoelastic region (LVR) which is described as a region where no structure breakdown of chocolate during measurement occurs (De Graef et al., 2011), can be measured. In this region, stress and strain are linearly proportional to each other and above the critical stress (or strain), structure breakdown occurs. The critical stress can be used to estimate the yield stress because structure breakdown is needed prior to the flow (De Graef et al., 2011; Taylor et al., 2009). Using this method, the particle-particle interaction at low shear stress can be better explained.

9.4.1.3.1 Flow behaviour

It can be seen in Table 9.5 that among palm sap sugar-sweetened chocolates, the chocolate which had the lowest moisture content (CPS VP60) exhibited the highest Cason yield value and the lowest Casson viscosity, whereas the chocolate which had the highest moisture content (CPS VP0) exhibited the lowest Casson yield value and the highest Casson viscosity. These results confirmed the previous finding (Chapter 7) that the presence of moisture which induced particle agglomeration decreased the Casson yield value but increased the Casson viscosity. In this case, it seemed that the surface area of the agglomerates was more dominant in determining Casson yield value than the surface area of individual particles, resulting in a lower Casson yield value. During shearing, the agglomerates could then be fragmented, creating smaller particles with "wet" surfaces, thus resulting in a higher Casson viscosity. Regarding the thixotropy value, CPS VP60 exhibited relatively low thixotropy value. This showed that CPS VP60 underwent effective moisture reduction during processing than CPS VP10 and CPS VP0, resulting in a more homogeneous chocolate. Eventhough the Casson viscosity and thixotropy value of CPS VP60 were still higher than those of CS Ref, CPS VP60 had a comparable Casson yield value ($p<0.05$) to that of CS Ref. Hence, by adjusting the duration of the vacuum process, palm sap sugar-sweetened chocolate with desired flow properties, can to some extent be produced. This method can be applied to replace the use of more cocoa butter, which is expensive, to lower the viscosity.

Table 9.5 Flow behaviour of chocolates produced with different vacuum duration

Chocolates	Flow parameter		
	Casson yield value (Pa)	Casson viscosity (Pa.s)	Thixotropy (Pa)
CPS VP0	0.3 ± 0.0^a	5.9 ± 0.0^c	4.7 ± 0.1^c
CPS VP10	1.3 ± 0.1^b	6.1 ± 0.1^c	5.9 ± 0.9^d
CPS VP60	10.4 ± 0.2^c	3.6 ± 0.1^b	2.9 ± 0.7^b
CS Ref	10.1 ± 0.3^c	2.4 ± 0.2^a	1.1 ± 0.5^a

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

9.4.1.3.2 Stress sweep

It can be observed in Figure 9.1 that CPS VP60 had a longer LVR and slightly higher complex modulus than CPS VP10 and CPS VP0, consecutively. This indicated that CPS VP60 had a more rigid chocolate matrix than the other palm sap sugar-sweetened chocolates. This phenomenon occurred due to the fact that CPS VP60 had a lower moisture content thus a lower degree of particle agglomeration and more particle-particle interaction were obtained, strengthening the chocolate matrix. The presence of higher degree of particle agglomeration in CPS VP10 and CPS VP0 may behave as large particles which reduced the particle-particle interaction, resulting in shorter LVR. Compared to the sucrose sweetened chocolate (CS Ref), as can be seen in the same figure, the complex modulus of CPS VP60 was slightly higher, but the LVR of CPS VP60 was shorter. The fact that CS Ref had smaller particle size D(v, 0.9) than CS VP60 (measure without sonication pre-treatment) may explain this observation.

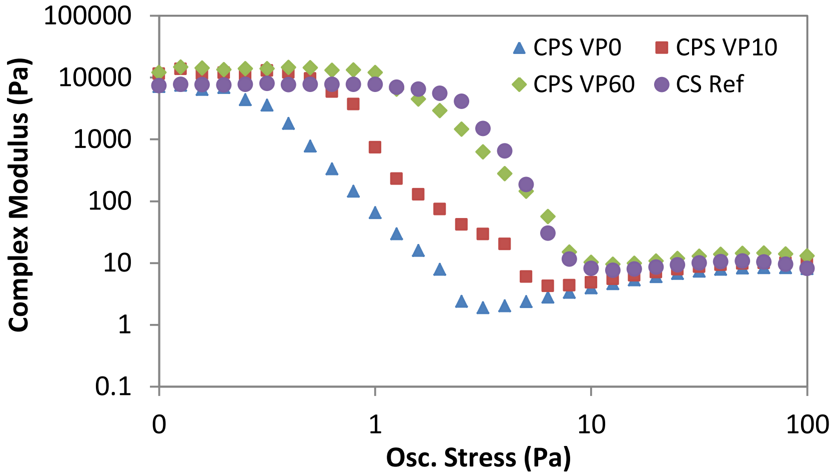

Figure 9.1. Stress sweeps of palm sap sugar sweetened chocolates produced with different vacuum duration

9.4.1.4 Microstructural Visualisation

To further elucidate the results obtained by the above-mentioned analyses, microstructure evaluation was carried out using polarised and normal light microscopy (Figure 9.2 and 9.3). The first method was used to visualise the crystallinity of the sugar in the chocolate suspension, while the second one was used to observe the agglomeration of the particles. Chocolate morphology visualisation using Scanning Electron Microscopy (SEM) was not applied in this experiment because based on the previous study (Chapter 7), the differences among samples could not be clearly observed, which was attributed to the relatively high total fat content of the samples.

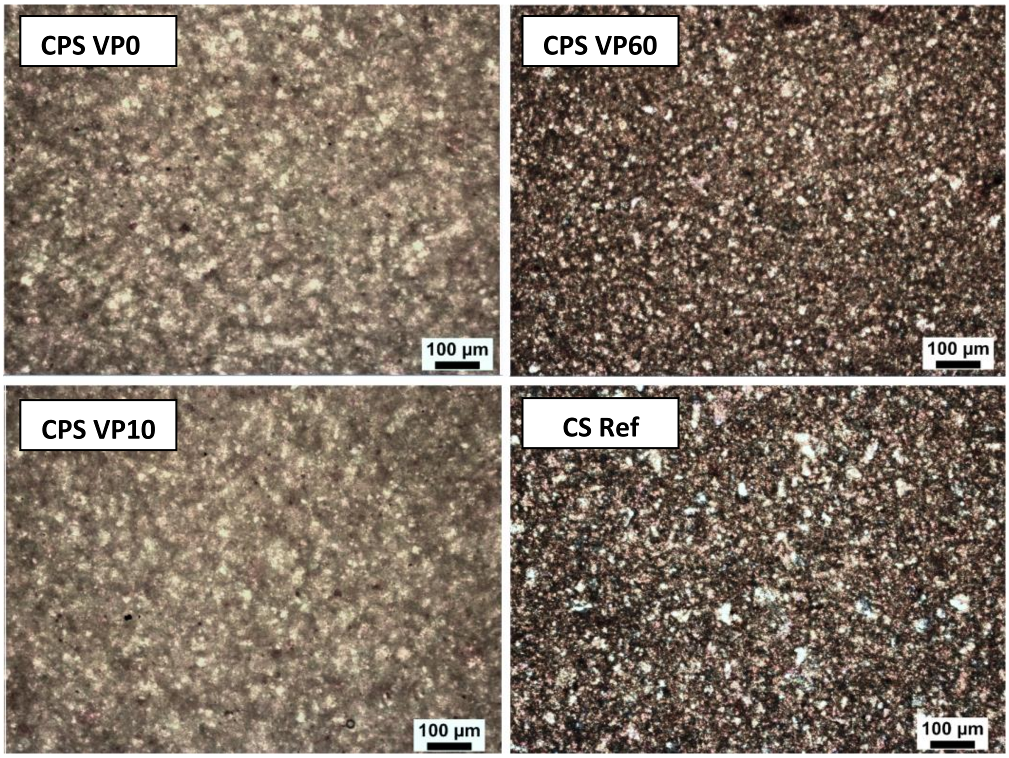

Figure 9.2. Micrographs of molten chocolates obtained with polarised light microscopy. The scale bar represents 100 µm.

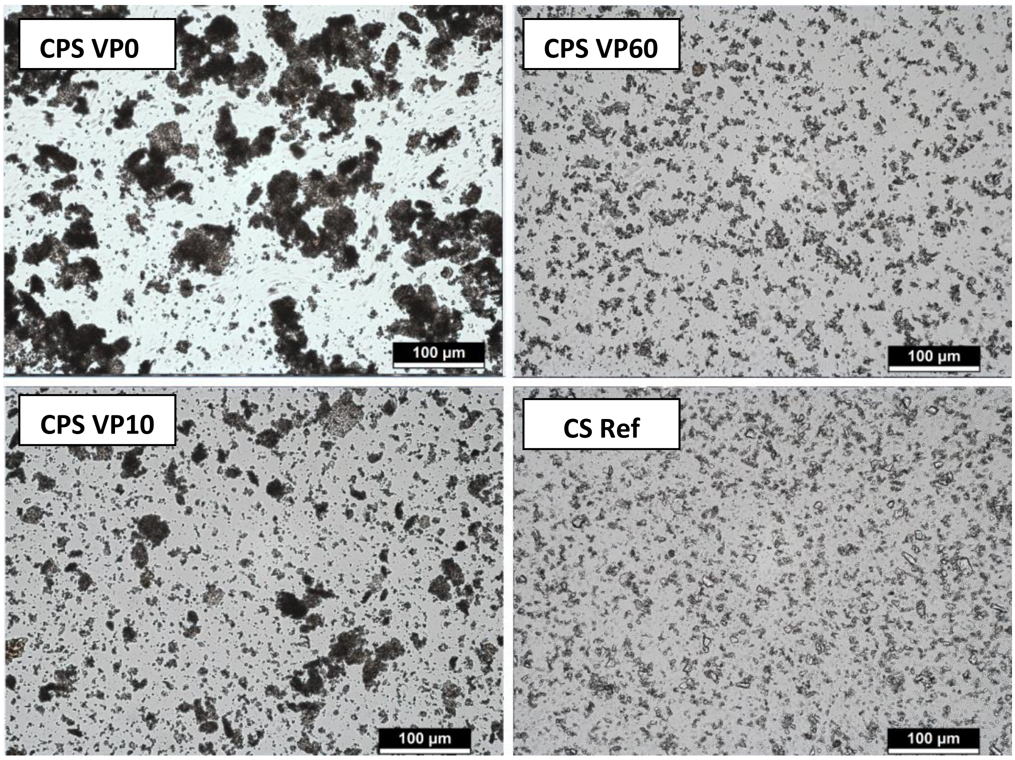

Figure 9.3. Particle agglomeration in isopropanol of chocolates obtained with normal light microscopy. The scale bar represents 100 µm.

As seen in Figure 9.2, polarised light microscopy revealed that palm sap sugar sweetened chocolate produced with the longest application of vacuum (CPS VP60) exhibited relatively clear sugar crystals with sharp/clear edges (whitish appearance). This was not observed in palm sap sugar sweetened chocolate produced without application of vacuum pump (CPS VP0) and with application of vacuum only for 10 min (CPS Ref), which tended to have unclear overlapping whitish edges, indicating some possible connections among individual sugar particles and/or agglomerates. This phenomenon occurred due to the presence of more amorphous part in the surface of palm sugar crystals. It can also be seen in Figure 9.2 that the microstructure of CPS VP60 was almost similar to that of sucrose sweetened chocolate (CS Ref), indicating that the presence of less moisture content during milling retained the formation of more amorphous part in the sugar surface, thus reducing the possibility of agglomeration.

Normal light microscopy revealed that the use of long duration of vacuum pump reduced the particle agglomeration in the chocolate. It can be seen in Figure 9.3 that CPS VP60 exhibited well-dispersed particles (black colour) in isopropanol (grey colour). This observation was almost similar to that of chocolate sweetened with sucrose (CS Ref). However, the presence of agglomerates increased when the duration of vacuum process was decreased (Figure 9.3), with the highest degree of agglomeration was observed in CPS VP0. These images confirmed the importance of sonication pre-treatment to measure the actual size of the chocolate particles.

9.4.2 LECITHIN VARIATION

9.4.2.1 Moisture content and melting profile of sugar phase in chocolate

Table 9.6 shows that irrespective of the lecithin concentration, all the chocolates sweetened with palm sap sugar (CPS0.6%, CPS0.7%, CPS0.8% and CPS0.4% Ref) exhibited relatively comparable moisture content in the range of 0.8 - 0.9%. This phenomenon can be explained by the fact that moisture evaporation only took place during dry mixing / dry conching-like step (Chapter 5) where no lecithin was added. Similar to the excess fat, lecithin present in the early step of dry conching-like process will optimally coat the solid particles, thus the removal of moisture become more difficult (Beckett, 2009). Therefore, the lecithin was added prior to the wet mixing/wet conching-like process before milling process and at the beginning of liquefaction, after milling process. In these stages, moisture reduction did not occur anymore.

Table 9.6 Moisture content and melting parameters of sugar phase in dark chocolates produced with different lecithin concentration

Chocolates	Moisture content (%)	Melting parameters			
		Onset (°C)	Peak Max (°C)	Width (°C)	Enthalpy (J/g)
CPS0.6%	0.9 ± 0.0^{a}	147.9 ± 0.3^{b}	164.5 ± 0.3^{a}	13.2 ± 0.5^{b}	47.2 ± 1.0^{b}
CPS0.7%	0.8 ± 0.1^{a}	147.9 ± 0.9^{b}	164.6 ± 0.5^{a}	12.7 ± 0.4^{b}	44.2 ± 1.0^{a}
CPS0.8%	0.9 ± 0.0^{a}	147.8 ± 0.3^{b}	164.3 ± 0.1^{a}	12.6 ± 0.2^{b}	44.6 ± 1.2^{a}
CPS0.4% Ref	0.9 ± 0.0^{a}	145.4 ± 0.6^{a}	165.1 ± 0.7^{a}	18.7 ± 0.7^{c}	50.7 ± 1.7^{c}

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

The moisture and impurities present in the palm sap sugar-sweetened chocolates act as plasticiser. Thus, compared to sucrose-sweetened chocolate which had very low moisture content and impurities, palm sap sugar-sweetened chocolate exhibited lower onset, melting peak temperature and enthalpy of sugar phase in chocolate (Section 9.4.1.1). The enthalpy value of CPS0.6% were slightly higher than that of CPS0.7% and CPS0.8% (Table 9.6), however, the onset, width and melting peak temperature of CPS0.6%, CPS0.7%, and CPS0.8% were comparable. The similarity of the values may be due to a relatively similar amount of moisture present in the chocolate. Compared to the CPS0.4% Ref chocolate; CPS0.7%, CPS0.6% and CPS0.8% had a melting profile with a slightly higher onset, but lower width and enthalpy values. This phenomenon may be attributed to the slightly higher degree of agglomeration in CPS0.4% (Table 9.7), thus resulting in a different breakage and melting behaviour.

9.4.2.2 Particle size distribution

It can be seen in Table 9.7 that CPS0.6%, CPS0.7% and CPS0.8% measured with or without sonication pre-treatment had a comparable D(v,0.9), D(v,0.5), D(4,3), D(3,2) and span values. While compared to CPS 0.4% Ref, they exhibited a slightly lower D(v,0.9), D(v,0.5), D(4,3), D(3,2) and span values. As expected, this phenomenon showed that lecithin concentration did not affect the particle size distribution of the chocolates. This result can be explained by the fact that during milling, the concentrations of lecithin were the same, thus possible different breakage behaviour due to different amount of lecithin did not occur.

Table 9.7 Particle size distribution of chocolates produced with different lecithin concentration

PSD (without sonication pre-treatment)

Chocolates	Distribution Percentiles (µm)			Derived Diameter (µm)		Span (-)
	D(v, 0.9)	D(v, 0.5)	D(v, 0.1)	D(4,3)	D(3,2)	
CPS0.6%	21.2 ± 0.5^{aA}	6.3 ± 0.1^{aA}	1.3 ± 0.0^{aA}	9.2 ± 0.2^{aA}	2.4 ± 0.1^{aA}	3.2 ± 0.0^{abA}
CPS0.7%	21.9 ± 0.4^{aA}	6.4 ± 0.2^{aA}	1.3 ± 0.0^{aA}	9.4 ± 0.2^{aA}	2.5 ± 0.1^{aA}	3.2 ± 0.1^{abA}
CPS0.8%	21.2 ± 0.2^{aA}	6.2 ± 0.1^{aA}	1.3 ± 0.0^{aA}	9.2 ± 0.1^{aA}	2.4 ± 0.1^{aA}	3.2 ± 0.1^{abA}
CPS04% Ref	26.2 ± 0.8^{bA}	7.4 ± 0.2^{bA}	1.4 ± 0.1^{abA}	11.1 ± 0.3^{bA}	2.7 ± 0.2^{abA}	3.4 ± 0.1^{bA}

PSD (with sonication pre-treatment)

Chocolates	Distribution Percentiles (µm)			Derived Diameter (µm)		Span (-)
	D(v, 0.9)	D(v, 0.5)	D(v, 0.1)	D(4,3)	D(3,2)	
CPS0.6%	16.1 ± 0.2^{aB}	5.5 ± 0.1^{aB}	1.1 ± 0.0^{aB}	7.3 ± 0.1^{aB}	2.1 ± 0.1^{aB}	2.7 ± 0.1^{bB}
CPS0.7%	16.3 ± 0.2^{aB}	5.7 ± 0.1^{aB}	1.1 ± 0.0^{aB}	7.5 ± 0.1^{aB}	2.1 ± 0.1^{aB}	2.7 ± 0.1^{bB}
CPS0.8%	16.4 ± 0.2^{aB}	5.7 ± 0.1^{aB}	1.2 ± 0.1^{aA}	7.5 ± 0.1^{aB}	2.3 ± 0.2^{aA}	2.7 ± 0.1^{bB}
CPS0.4% Ref	16.6 ± 0.6^{aB}	6.2 ± 0.2^{bB}	1.4 ± 0.2^{bA}	7.8 ± 0.2^{bB}	2.7 ± 0.2^{bA}	2.5 ± 0.2^{abB}

Mean values ± standard deviations from triplicate analysis.
Different superscripts (lowercase) in the same column indicate significant differences ($p < 0.05$) among samples, different superscripts (uppercase) in the same sample with different treatments (with and without sonication pre-treatment) indicate significant differences ($p < 0.05$) among samples

Regarding the degree of agglomeration, it can be seen in Table 9.7 that all palm sap sugar-sweetened chocolates exhibited relatively low degree of agglomeration. This phenomenon was due to the fact that all the chocolates were produced with 60 min of vacuum process. In the previous section (Section 9.4.1), long duration of vacuum process was proven to be more effective in removing moisture.

9.4.2.3 Rheological behaviour

9.4.2.3.1 Flow behaviour

It can be seen in Table 9.8 that the Casson yield value of palm sap sugar sweetened chocolates increased as the lecithin concentration was increased, while the Casson viscosity and thixotropy among samples were comparable. Since all the chocolates had a comparable particle size and moisture content, the increase of Casson yield value with the increase of lecithin concentration could be attributed to the formation of reverse micelles in cocoa butter and/or the formation of self-association of lecithin possibly as multilayers around sugar (Afoakwa et al., 2007; Beckett, 2009). This result is in agreement with the study carried out by Schantz and Rohm (2005a). They reported that the addition of more than

0.4% lecithin into chocolate with fat and moisture content of 31% and 0.4%, respectively, resulted in an increase in Casson yield value, while Casson viscosity decreased.

At low stresses, the flow behaviour is mainly dominated by particle–particle interactions (Mongia and Ziegler, 2000). Thus, the smaller the particle size, the larger the specific surface area and the stronger the particle-particle interactions are, resulting in a higher yield values. In palm sap sugar sweetened chocolates (CPS0.6%, CPS0.7%, CPS0.8% and CPS0.4% Ref), particle agglomeration occurred. In this case, the surface area of the agglomerates was more dominant than that of the individual particles in determining the yield value. However, once the flow was started, the agglomerates may be fragmented, thus smaller individual particles with higher total surface area were created. Consequently, more cocoa butter and/or lecithin were needed to maintain the viscosity. Therefore, the increase of Casson viscosity due to the formation of reverse micelles and/or self-association of lecithin, which commonly take place due to the presence of excessive of lecithin, did not occur. The phenomenon that the excess lecithin did not increase the Casson viscosity, in contrast to Casson yield value, was also reported by Schantz and Rohm (2005a), Beckett (2009), (Afoakwa, 2016), but no clear reasons were provided.

Table 9.8 Flow behaviour of chocolates produced with different lecithin concentration

Chocolates	Flow parameter		
	Casson yield value (Pa)	Casson viscosity (Pa.s)	Thixotropy (Pa)
CPS0.6%	12.7 ± 0.3[b]	2.0 ± 0.1[a]	1.8 ± 0.5[ab]
CPS0.7%	14.1 ± 0.2[c]	2.2 ± 0.1[a]	2.1 ± 0.6[b]
CPS0.8%	17.3 ± 0.2[d]	2.1 ± 0.0[a]	2.1 ± 0.5[b]
CPS0.4% Ref	10.4 ± 0.2[a]	3.6 ± 0.1[c]	2.9 ± 0.7[c]

Mean values ± standard deviations from triplicate analysis.
Different superscripts in the same column indicate significant differences ($p < 0.05$) among samples

Compared to CPS0.4% Ref, as can be seen in Table 9.8, palm sap sugar sweetened chocolates with higher lecithin concentration (CPS0.6%, CPS0.7%, CPS0.8%) had significantly higher ($p<0.05$) Casson yield value, but lower Casson viscosity and thixotropy. This showed the effectiveness of lecithin in maintaining the viscosity parameters of palm sap sugar sweetened chocolate. Moreover, compared to sucrose-sweetened chocolate (CS Ref) (Table 9.5), it can be seen in Table 9.8 that CPS0.6%, CPS0.7%, and CPS0.8% had a comparable

Casson viscosity. The results suggested that the adjustment of lecithin concentration can to some extent be used to tackle the viscosity issue in palm sap sugar sweetened chocolate. In this study, 0.6% of lecithin was the best compromise among Casson yield value, Casson viscosity and thixotropy for palm sap sugar-sweetened chocolate with fat content of 36%.

In practice, to adjust the lecithin concentration in palm sap sugar sweetened chocolate, the chocolate application should be taken into account. For instance, for the moulded chocolate bar production and cookie enrobing application, chocolate with relatively low Casson yield value is needed. Hence, the lecithin added should not be too much; otherwise the Casson yield value may increase. In chocolate coated cookie, the agglomerates present in chocolate, indicated by relatively high thixotropy value, will be masked by the grittiness of the cookie during consumption. In the case that the molten chocolate is transported from one container to other containers through piping system, low Casson viscosity chocolate is required. Thus, the addition of lecithin is recommended to ensure that the molten chocolate has the desired flow properties.

9.4.2.3.2 Stress sweep

It can be observed in Figure 9.4 that the LVR of CPS0.6%, CPS0.7% and CPS0.8% were comparable. This phenomenon indicated that the difference of 0.1% lecithin concentration among chocolates did not significantly influence the rigidity of the chocolates matrix. However, compared to CPS0.4% Ref, CPS0.6%, CPS0.7% and CPS0.8% had a longer LVR which can be explained by the fact that CPS0.4% had slightly bigger particle size D(v, 0.9) (Table 9.7) than CPS0.6%, CPS0.7% and CPS0.8%, thus less particle-particle interaction. The possibility of reverse micelle formation and/or self-association formation of lecithin possibly as multilayers around sugar in CPS0.6%, CPS0.7% and CPS0.8%, due to the excess lecithin, which reduced the effectiveness of lecithin, may also contribute to this phenomenon. This phenomenon was indicated by the increase in Casson yield value as the lecithin concentration was increased (Table 9.8). This result was in agreement with the study conducted by De Graef et al. (2011). In their work, an addition of 0.2% lecithin into chocolate with a fat content of 36.7% caused the shortening of LVR, while the addition of a higher lecithin concentration (0.4%, 0.6%, 0,8%) resulted in an increase in LVR.

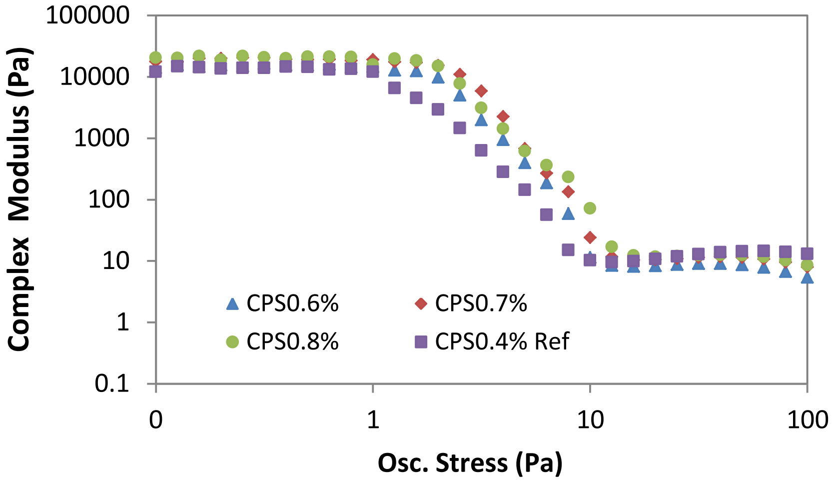

Figure 9.4. Stress sweeps of palm sap sugar sweetened chocolates produced with different lecithin concentrations

9.4.2.4 Microstructural visualisation

Microstructural visualisation was carried out to further explain the results of the previous analyses. Two microscopy methods, similar to the ones used in the application of vacuum experiment, namely polarised and normal light microscopy were employed. The use of polarised light microscopy to visualise the sugar crystals in CPS0.6%, CPS0.7% and CPS0.8% as well as CPS0.4% Ref resulted in the observation of relatively similar images with sharp-edge sugar crystals (whitish appearance). Typical images of the palm sap sugar sweetened chocolates (CPS0.6%, CPS0.7%, CPS0.8% and CPS0.4% Ref) can be seen in Figure 9.5 (A). Regardless of the lecithin concentration, the use of normal light microscopy revealed that all palm sap sugar sweetened chocolates exhibited relatively well-dispersed particles (black colour) in isopropanol (grey colour). Typical images of the CPS0.6%, CPS0.7%, CPS0.8% and CPS0.4% Ref can be seen in Figure 9.5 (B). Eventhough the lecithin concentration affected the rheological behaviour of chocolates (Section 9.4.2.3), the clear influence of lecithin in microstructure of palm sap sugar-sweetened chocolates could not be visualised using polarised and normal light microscopy.

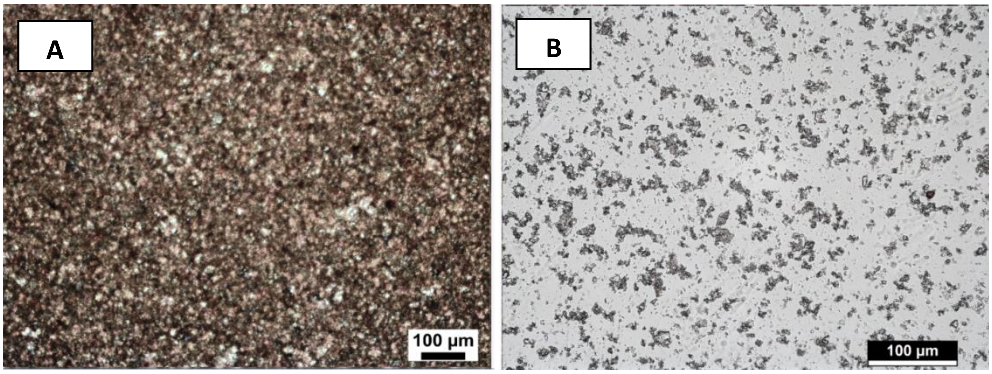

Figure 9.5. Typical microstructural images of chocolates sweetened with palm sap sugar produced by alternative processing using 60 min vacuum process. (A) Micrograph of molten chocolate obtained with polarised light microscopy. (B) Particle agglomeration in isopropanol of chocolates obtained with normal light microscopy. The scale bar represents 100 µm.

9.5 CONCLUSIONS

Formula and processing method modification which can be easily and feasibly applied by small-scale chocolate producer is very important to create palm sap sugar sweetened chocolate with desired characteristics. This study showed that by adjusting the lecithin concentration and duration of vacuum process during dry mixing / dry conching-like step, the chocolate viscosity parameters can be maintained. In the addition of lecithin in chocolate suspension, the application of the chocolates should be considered, e.g. as a solid-eating chocolate or as an intermediate product which will be further applied in various foods. Thus, the viscosity parameters (Casson yield value, Casson viscosity, thixotropy) can be optimally adjusted. From this work, it can be seen that by increasing the lecithin concentration, the Casson viscosity and thixotropy decreased, while the Casson yield value increased due to the formation of micelle and/or self-association of lecithin possibly as multilayers around sugar.

Regarding the application of vacuum during dry mixing / conching-like step, it can be concluded that the longer the duration of vacuum process, the higher the amount of moisture that can be evaporated, which resulted in lower viscosity. However, in practice, the use of vacuum process for longer duration may interfere with the aroma development of chocolate. This limitation should be considered when using vacuum pump in the chocolate production.

The outcome of this work will be very useful for the small-scale palm sap sugar-sweetened chocolate producers. By process adaptation of the studied small-scale production system and fine-tuning of lecithin concentration, flow properties of palm sap sugar-sweetened chocolates similar to those of sucrose-sweetened chocolate can be obtained. This is the first step towards development of such a system. Further research is needed as taste was not considered.

CHAPTER 10

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

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CHAPTER 10
GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

10.1 GENERAL CONCLUSIONS

The use of palm sap sugar as sweetener for traditional beverages and foods contribute not only to the sweetness but also to the development of colour, taste and aroma. Hence, its use in chocolate was also expected to have similar impact. In order for small-scale cocoa and/or palm sap sugar farmers to easily adopt this practice, a small-scale alternative chocolate processing method involving a combination of ball mill and Stephan mixer was used throughout this study.

The physicochemical properties of palm sap sugar are highly influenced by sap/nectar used for the sugar production. These properties are affected by variety of palm tree, maturity of the inflorescence, climatic condition, soil fertility, timing and duration of the sap tapping as well as possible sap fermentation that occurs during storage. In addition, different processing techniques applied by farmers, *e.g*. the duration and temperature result in the variation of physicochemical characteristics of palm sap sugar. Combination of time and temperature are the factors influencing the degree of Maillard reaction that occurs during sugar production. The degree of Maillard reaction, then, influences the physicochemical properties of palm sap sugar, including sucrose, fructose and glucose content as well as aroma profile and colour of the sugar.

Coarse palm sugars used in this study contained several impurities, such as moisture content, protein, fructose, glucose and ash. These impurities, especially the moisture content which influences the presence of amorphous part, lower the melting and glass transition temperature of palm sap sugars. Moreover, the presence of amorphous part in combination with moisture content formed layers which make the sugar surface stick to each other, creating particle agglomerations. This phenomenon, to some extent, influences the particle density and the particle size distribution of palm sap sugars.

Ball mill is a promising small-scale chocolate processing machine that can be easily and economically applied by small-scale chocolate makers in cocoa producing countries.

However, since ball mill has limitation in effectively removing moisture from the chocolate mixture, utilisation of a liquefier and/or moisture removing device is needed. By using this device, a lower moisture and more homogeneous chocolate suspension can be achieved.

The combination of ball mill and liquefier or moisture removing device, called alternative processing, was less effective in removing moisture compared to the conventional one, which consists of a sequence of mixing, roll refining, and conching. Hence, the degree of agglomeration of palm sap sugar-sweetened chocolate produced by alternative processing was higher than that of chocolate produced by conventional processing. Moisture content in combination with glucose and fructose as hygroscopic materials were responsible for the formation of agglomerates in chocolate suspension. Moreover, they also triggered the formation of more amorphous sugar during refining. Thus, palm sap sugar sweetened chocolate produced by alternative processing had a lower ratio of enthalpy, an indicator of the presence of crystalline part, compared to that of palm sugar-sweetened chocolate produced by conventional processing. Due to this phenomenon, palm sap sugar sweetened chocolate produced by alternative processing had lower Casson yield value and higher Casson viscosity than palm sap sugar sweetened chocolate produced by conventional processing. In terms of aroma profile, the concentration of the volatiles in palm sap sugar produced by alternative processing was generally significantly higher than that in palm sap sugar produced by conventional processing. This can be explained by the presence of a higher amount of amorphous sugar which absorbs some of the volatiles resulting in a more intense flavour chocolate.

The presence of the particle agglomeration influences the particle-particle interaction in the chocolate suspension which in turn affects the quality attributes of chocolates. In molten chocolate, particle-particle interaction determines the flow behaviour of chocolate, mainly its Casson yield value. The smaller the particle size, the larger is the specific surface area. Thus the stronger the particle-particle interaction becomes, and therefore resulting in higher yield values. Due to the presence of agglomerates, the Casson yield value of palm sap sugar sweetened chocolate was highly influenced by the surface area of the agglomerates. Thus, the Casson yield value of palm sap sugar sweetened chocolate was lower than that of sucrose sweetened chocolate. However, the Casson viscosity of palm sap sugar sweetened chocolate was higher than the Casson viscosity of sucrose sweetened chocolate. During shearing, the agglomerated particles might be fragmented, resulting in smaller particles with higher and "wet" surfaces, leading to a higher viscosity. Thixotropy is a parameter that can be used to evaluate the presence of agglomerates. Therefore, due to the presence of agglomerates, the thixotropy value of palm sugar sweetened chocolate was higher than that of sucrose sweetened chocolate.

Particle-particle interaction in well-tempered chocolate determines the colour and hardness of chocolate. Due to its lower particle density, palm sap sugar exhibited a higher particle volume fraction in the chocolate matrix. Therefore, an increased particle-particle interaction occurred in palm sap sugar sweetened chocolate resulting in a lighter colour and a harder chocolate. Chocolate with more particle-particle interaction tend to be denser and able to scatter more light, resulting in a lighter colour. Some variations on the colour values can be associated with the smoothness/roughness of the chocolate surface. A different level of surface roughness scatter light distinctively, thus resulting in a different colour parameters values recorded by colorimeter. Chocolate formulated with palm sap sugars contained higher moisture and amorphous sugar level than chocolate sweetened with sucrose. This may create "stronger" sugar networks in the chocolate, thus resulting in a higher force required to penetrate the chocolate. This explains the higher hardness values recorded for chocolates formulated with palm sap sugar.

The use of palm sap sugar has potential for the development of dark chocolate with a distinctive aroma profile. The findings showed that the aroma profile of palm sugar highly influenced the aroma profile of dark chocolate, which could be marked with a higher concentration of some alkyl pyrazines, alkyl furans, alkyl alcohols, 2-acetylpyrrole, and acetic acid observed in the palm sugar sweetened chocolate. Moreover, there were some volatiles present in palm sugar which were only detected in palm sugar sweetened dark chocolate, such as 2-furoic acid, 3-hydroxy-2-butanone, 1-hydroxy-2-propanone, 2,3-dihydro-5-dihydroxy-6-methyl-4H-pyran-4-one, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP). The high concentration of those volatiles in dark chocolate can be attributed to the presence of a higher amount of amorphous part present in palm sap sugar sweetened chocolate. During chocolate production, the amorphous part was able to absorb the volatiles, thus resulting in a chocolate with more intense aroma.

The use of palm sap sugar as chocolate sweetener created chocolates with a distinctive aroma profile which is a typical aroma of foods sweetened with palm sap sugar. However, the use of this sugar also resulted in chocolate with relatively high degree of agglomeration which affects the fineness and rheological behaviour of chocolate. Therefore, approaches that can be easily and feasibly applied by small-scale chocolate producer to overcome this problem, such as modification of processing method by extending the duration of vacuum process during dry mixing and/or modification of formulation by increasing the lecithin concentration, are highly required. Even though the improvement of the chocolate quality was focused on the fineness and rheological behaviour of chocolate, other quality attributes, particularly the appearance and hardness can also be improved at the same time, to some extent. This is possible since their values are also influenced by the same factors, *i.e.* particle size distribution, fat content, moisture content and particle density.

The use of maximum vacuum duration during dry mixing/dry conching-like process was very effective in evaporating moisture, which resulted in a lower degree of agglomeration, Casson viscosity and thixotropy. Modification of formulation by adding a higher lecithin concentration also decreased the degree of agglomeration in palm sap sugar-sweetened chocolate. In terms of rheological behaviour, the higher the lecithin concentration, the higher the Casson yield value became, due to the occurrence of reverse micelle and/or the formation of self-association of lecithin possibly as multilayers around sugar. Regarding the Casson viscosity and the thixotropy, their values decreased to some extent as the lecithin concentration was increased. Therefore, the decision to add more lecithin should consider the application of chocolate.

9.2 FUTURE PERSPECTIVES IN PALM SAP SUGAR-SWEETENED CHOCOLATE RESEARCH

Findings from this work provide knowledge about the functionality of palm sap sugar in dark chocolate as well as small-scale approach for production of palm sap sugar sweetened chocolate. These findings can be applied by farmers and small chocolate makers to produce palm sap sugar sweetened chocolate with the desirable characteristics. Thus, by producing their own chocolate, cocoa and palm sap sugar farmers can earn more economic benefits.

In term of scientific work, there are several points that still need to be done in the future studies in order to obtain comprehensive understanding about this topic. The points are as follows:

1) **Conducting sensory evaluation.**

   A consumer test is required to investigate the acceptance of palm sugar-sweetened chocolate in the market. In addition, a Quantitative Descriptive Analysis (QDA) test is also needed to objectively describe the sensory characteristics of palm sap sugar-sweetened chocolate. To obtain reliable results, QDA test should be done by trained panellists.

2) **Conducting a stability test on the palm sap sugar-sweetened chocolate.**

   Palm sap sugar is highly produced and consumed in the Southeast Asian region. Thus, this type of chocolate is expected to be suitable for the Southeast Asian population. Considering that this region has hot temperature and high humidity, thermal stability test is highly required. In this experiment, the extent of sugar network in stabilising the chocolate matrix can be investigated.

3) **Conducting a study about impact of vacuum process on the aroma development of palm sap sugar sweetened chocolate.**

   The application of vacuum process during dry mixing successfully evaporates more moisture in the production of palm sap sugar sweetened chocolate. However, during this process, the formation of chocolate aroma may be interfered. Thus, in order to create chocolate with the desired aroma profile, an experiment about the impact of vacuum process which can be combined with other variables, such as dry mixing temperature, *etc.*, can be carried out.

4) **Investigating the formation of amorphous sugar in palm sap sugar-sweetened chocolate.**

Amorphous sugar highly affects the quality attributes of palm sap sugar sweetened chocolate, more particularly fineness, flow behaviour, hardness and aroma. Thus, to be able to effectively control its effects, quantitative analyses of amorphous sugar in each step of chocolate production are required. The investigation of amorphous sugar can be done using Atomic Force Microscopy (AFM).

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PUBLICATIONS

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    - **Saputro, A. D.**, Van de Walle, D., Kadivar, S., Sintang, M. D. B., Van der Meeren, P., Dewettinck, K. (2017). Investigating the rheological, microstructural and textural properties of chocolates sweetened with palm sap-based sugar by partial

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- **Submitted manuscripts and manuscripts under preparation for submission**
  - **Saputro, A.D.,** Van de Walle, D., Dewettinck, K. Physicochemical properties of palm sap sugars from Indonesian origin.
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- **Oral presentations at conference and Workshop**
  - **Saputro, A.D.** The role of sugar, alternative sweeteners and bulking agents in the quality attributes of chocolate. The 5th Edition of Cocoa and Chocolate Workshop, 2017, Ghent, Belgium.
  - **Saputro, A.D.,** Van de Walle, D., Dewettinck, K. Impact of sucrose and palm sugar blend as sweetener on the quality attributes of dark chocolate. IUFoST 2016: 18th World Congress of Food Science and Technology. Dublin, Ireland

- **Saputro, A. D.,** Van de Walle, D., De Clercq, N., Amoafo Mensah, M., Van Durme, J., Dewettinck, K. Quality attributes of palm sugar containing chocolate using alternative processing. The 3rd International congress on Cocoa Coffee and Tea (COCOTEA 2015), Aveiro, Portugal.

- **Poster presentation at conference**
    - **Saputro, A. D.,** Van de Walle, D., Dewettinck, K. Palm sap-based sugar and its potency as chocolate sweetener. The 21st National symposium on Applied Biological Sciences (NSABS 2016).
    - **Saputro, A. D.,** Van de Walle, D., González, M.P., Dewettinck, K. Controlling the flow behaviour of dark chocolate formulated with coconut sugar. The 22nd National symposium on Applied Biological Sciences (NSABS 2017).

MASTER STUDENT SUPERVISION

- Michael Amoafo Mensah (2014/2015). The use of coconut and palm sugar as chocolate sweetener.
- Bobby Antan Caiquo (2014/2015). Impact of refining conditions on the flow behaviour of chocolate.
- Maria Paulina González (2015/2016). Flow behaviour of coconut sugar sweetened chocolate.

DOCTORAL TRAINING PROGRAM (DTP) RELATED TO RESEARCH

- Differential Scanning Calorimetry (DSC) Training
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