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Interactions of fat migration and crystallization in filled dark chocolate products

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Abstract

A white layer on the surface of chocolates, the so-called fat bloom, is the decisive parameter for the quality loss of this product group. Filled products, such as pralines, are particularly susceptible. This is because in such filled products oils can migrate from the filling into the chocolate.
The *migrated oil*, which migrates from the filling into the chocolate during storage can change the crystalline structure of the chocolate. The migration mechanism has not yet been fully understood. Moreover, the influence of the initial oil has hardly been investigated. *Initial oil* is oil from the filling, which - during the production of the filled products - already gets into the liquid chocolate that later encloses the finished product as a shell. This oil can change the crystal state of the chocolate.
The microstructure of chocolate can be changed by adding particles, by adding vegetable oils or edible fats, and by changing the crystal state of the polymorphic cocoa butter. This can be used to retard the migration of oils from the filling into the chocolate and resulting fat bloom in filled chocolate products. Therefore, a model system was developed to study the influence of chocolate microstructure on the migration of oils from the filling through the chocolate. Finally, a realistic experiment with cold-stamped chocolates was conducted to verify the results.
The study shows that both the initial and migrated oil accelerate the transition from the less stable to the more stable crystal form of cocoa butter. Moreover, the migration is faster for samples with the most stable polymorphic crystal form β_{VI} . On the other hand, oil in the chocolate, whether initial oil or migrated oil, reduces the migration rate. Fat bloom formation was not accelerated by initial oil, as the effects on migration and crystallization of the initial oil appear to counteract to each other.

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Symbols

A	area
D_{eff}	effective diffusion coefficient
D_0	molecular diffusivity
g	gravitational acceleration
G	Gibbs free energy
h	height of capillary rise
H	enthalpy
l	length
m	mass
q_3	volume based particle density distribution
Q_0	quantitative cumulative particle size distribution
r	radius
S	entropy
t	time
T	temperature
V	volume
γ	surface energy
μ	viscosity of the fluid
Φ	volume fraction
ρ	density of the fluid

σ	surface tension
τ	tortuosity
Θ	contact angle

Abbreviations

AMF	anhydrous milk fat
BET	Brunauer Emmett Teller
CBE	cocoa butter equivalent
CBR	cocoa butter replacer
CBS	cocoa butter substitute
CE	circle equivalent
CLSM	confocal laser scanning microscopy
DSC	differential scanning calorimetry
ELSD	evaporative light scattering detector
FA	fatty acid
FFA	free fatty acid
FID	flame ionization detector
GC	gas chromatography
HPLC	high performance liquid chromatography
LLL	trilinolein
LLO	1,2-dilinoleoyl-3-oleoylglycerol
LOO	1-linoleoyl-2,3-dioleoylglycerol
MRI	Magnetic Resonance Imaging
NMR	nuclear magnetic resonance
OOO	triolein
POP	1,3-dipalmitoyl-2-oleoylglycerol
POS	1,3-palmitoyl-stearoyl-2-oleoylglycerol
RCF	relative centrifugal force
RR	recovery rate
SFC	solid fat content

SOS	1,3-distearoyl-2-oleoylglycerol
SSS	tristearin
TAG	triacylglyceride
TI	temper index
TMSH	methanolic trimethylsulfoniumhydroxide
WI	Whiteness Index
XRD	X-ray diffraction

1 Introduction

Filled chocolates are the most popular chocolate products amongst consumers. However, they are particularly susceptible to fat bloom, a white or grayish layer on the surface of the product. Fat bloom in filled chocolate is often assigned to migration of oil from the filling into the surrounding chocolate.
Even though migration in chocolate has been investigated for several decades, the mechanism is still unclear. Two main mechanisms for migration in chocolate are discussed. The first description of migration of oil molecules in chocolate was based on diffusion of these molecules as the transport mechanism (Ziegleder, Moser, et al. 1996a; Ziegleder, Moser, et al. 1996b; Galdámez et al. 2009; Ghosh et al. 2002; Lee et al. 2010; Maleky et al. 2012; K. L. McCarthy and M. J. McCarthy 2008; Motwani et al. 2011). In 2004 capillary flow was supposed as another possible mechanism to describe migration in chocolate (Aguilera et al. 2004; Choi, K. L. McCarthy, and M. J. McCarthy 2005; Guiheneuf et al. 1997). A combination of both mechanisms is also conceivable (Deka et al. 2006; Reinke, Hauf, et al. 2015; Rousseau and P. Smith 2008). A third less probable mechanism which is discussed is convective flow (Altimiras et al. 2007; Dahlenborg, Millqvist-Fureby, et al. 2015a; Loisel et al. 1997). All mechanisms highly depend on the microstructure of the chocolate, which is influenced by sugar, cocoa and eventually milk particles, but also the structure of the crystals of the cocoa butter, such as crystal density, crystal size or polymorphic form.
The crystal structure is affected by intrinsic and extrinsic factors. Intrinsic factors are triacylglyceride (TAG) composition, amount of minor components or presence of particles (Cebula and K. W. Smith 1992; Danzl and Ziegleder 2016a; Danzl and Ziegleder 2016b; Foubert 2003; Foubert, Vanrolleghem, Thas, et al. 2004; Patel and Dewettinck 2015; Ribeiro et al. 2015; K. W. Smith, Bhaggan, Talbot, and van Malssen 2011; Tietz and Hartel 2000; Sato 1999; Sato 2001; Svanberg et al. 2011a; Svanberg et al. 2011b). Extrinsic factors are especially production parameters, such as cooling time and temperature or introduced shear (Zeng 2000; Afoakwa, Paterson, M. Fowler, and Vieira 2008c; Augusto et al. 2012; Dhonsi and Stapley 2006; Guthrie 2008; Maleky 2015; Padar, Mehrle, et al. 2009; Ramel and Marangoni 2017; Shi and Maleky 2015; Stapley et al. 1999; Ziegleder 1991; Ziegleder 1993c; Ziegleder 1995; Ziegleder and Kegel 1989; Marty and Marangoni 2009). An extrinsic factor that causes intrinsic changes is the partial transfer of filling oils into the chocolate during the manufacturing process. This results in a changed TAG composition, which can alter crystallization (Rothkopf and Danzl 2015; Cebula and Ziegleder 1993).
The crystallization rate of the chocolate might be accelerated or decelerated by entrained filling oils, depending on their type. However, this requires an adjustment of the processing parameters. The amount of crystallized fat in the chocolate might also be reduced

by filling oils. The change of the crystallization behavior of chocolate with the amount of added filling oil depends on the TAG composition of the filling oil (Rothkopf and Danzl 2015). However, filling oils affect not only crystallization during production but also during storage. Migrating hazelnut oil is known to promote polymorphic transition of cocoa butter from the stable β_{V} into the more stable β_{VI} crystal form during storage (K. W. Smith, Cain, et al. 2007). The effects of initial filling oil are not well investigated and might differ from the effects caused by migrated filling oil.
Although most investigations focus on cocoa butter, the impact of particles in chocolate must be taken into account. Particles have an impact on crystallization (Svanberg et al. 2011a; Svanberg et al. 2011b; Afoakwa, Paterson, M. Fowler, and Vieira 2008c) as well as on migration (Altimiras et al. 2007; Dahlenborg, Millqvist-Fureby, et al. 2015a; Dahlenborg, Millqvist-Fureby, et al. 2015b).
Migration and crystallization in filled products cannot be regarded separately. *Initial oil*, which is already contained in the liquid chocolate might affect crystallization, which affects microstructure and thus migration. On the other hand, these already present filling oils reduce migration pressure due to the reduction of the concentration gradient. During storage initial or migrating filling oil affects the polymorphic transition. However, migration might also depend on the polymorphic form of the chocolate crystal lattice.
Several model systems were developed and used to investigate crystallization and migration separately. Further, the impact of crystallization on migration and of migration on crystallization was studied. Finally, a realistic model praline was used to investigate the interaction of migration and crystallization in several life-cycle steps.
The initial hypothesis of this work is that the crystalline state of cocoa butter in chocolate influences the migration of oils from the filling through the chocolate, e.g. in a praline. Additionally the migrating oil is assumed to alter the crystalline structure of the cocoa butter in chocolate. Therefore, the aim of this work was to identify impact factors for cocoa butter crystallization, oil migration in chocolate and their interaction.

2 Background

2.1 Chemical properties of fats and oils

The ingredients used in chocolate and chocolate products are cocoa products, namely cocoa liquor and cocoa butter, sugar and in case of milk chocolate, milk products, such as milk powder and milk fat. The amount of these components is regulated by law or defined in guidelines. While flavor and taste are mainly defined by cocoa solids and sugar, the physical properties and flavor release are determined by cocoa butter. Cocoa butter, as well as other edible fats and oils, is mainly composed of triacylglycerides (TAGs). Other components are mono- and diglycerides, free fatty acids (FFAs) and minor components such as phospholipids, phytosterols, tocopherols and hydrocarbons (Gunstone 2013). Fats and oils contain between 95 %(w/w) to 99 %(w/w) TAG depending on refining and amount of unsaponifiable material (Gunstone 2013). TAG are fatty acid (FA) esters of the trihydric alcohol glycerol and are shown in fig. 2.1 (Gunstone 2013).

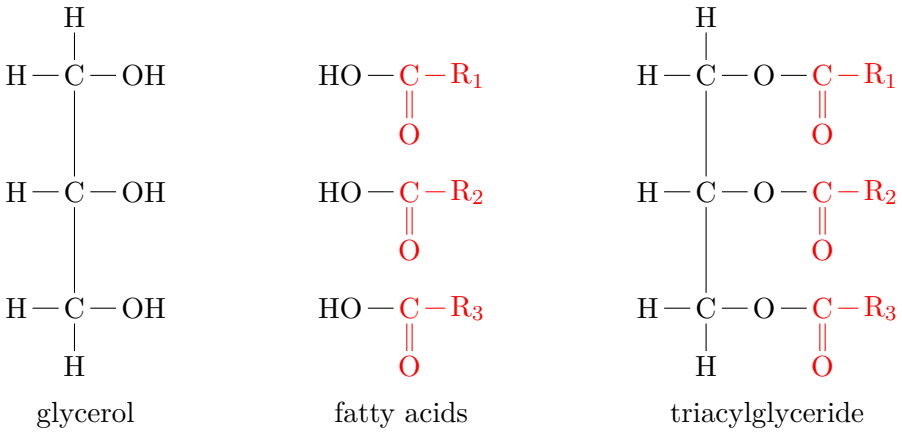


Figure 2.1: TAG are composed of glycerol and FAs with residual carbon chains R of different length and number of double bonds

Due to the chiral character of the carbon atom in position sn-2, three conformations for TAG are possible, shown in fig. 2.2 (Hall et al. 2008). While the trident structure is mainly found at oil/water interfaces, the tuning fork and chair conformation depend on the saturation and sn-position of FA-chains (Bayés-García et al. 2015).

FA in edible fats and oils are unbranched and include 4 to 26 carbon atoms. The degree of saturation of the FA determines the physical state at room temperature. Fats which contain a high amount of saturated FA are mainly solid, whereas oils, which

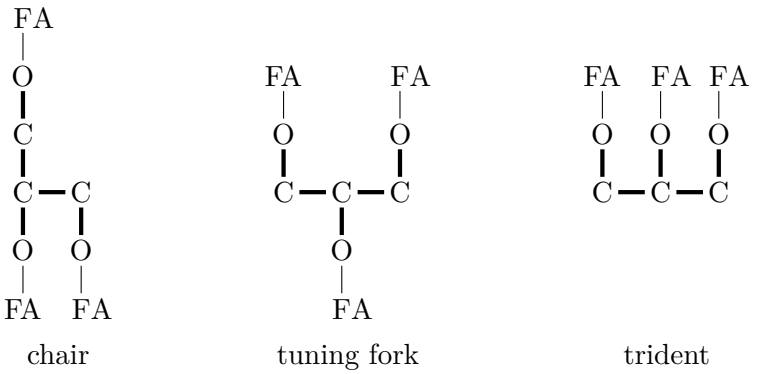


Figure 2.2: Different conformations of TAG with marked glycerol moiety

contain high amounts of unsaturated FA are liquid at room temperature. The amount of saturated and unsaturated FA in vegetable oils is mainly influenced by climate, especially temperature. Plants in cold growing areas include more unsaturated FA to ensure that the oil is liquid and transportable inside the plant. Since humans and animals are able to produce body heat and meet their requirements of unsaturated fats by food supply, their unsaturated FA biosynthesis is limited. (Krist 2013a)
Fig. 2.3 shows the main FAs, namely palmitic, oleic and stearic acid, which are present in almost every natural (vegetable and animal) oil or fat. It can be seen that the double bond in oleic acid causes a kink which affects TAG interaction.

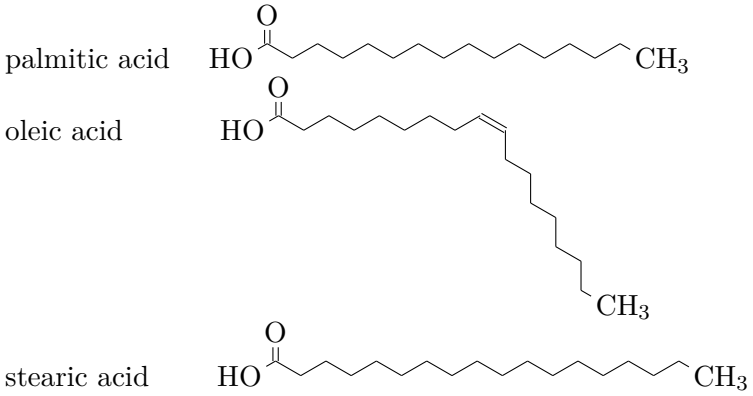


Figure 2.3: The main fatty acids in natural (vegetable and animal) oil or fat

2.1.1 Cocoa butter

In tab. 2.1 the main FAs in cocoa butter grown in different regions are listed.

The position of FA in TAG is not randomized in edible oils and fats. While saturated FA are at the end positions sn-1 or sn-3 of the glycerol, the sn-2 position is usually occupied by an unsaturated FA (Gunstone 2013). Therefore the main TAG in cocoa butter

Table 2.1: Fatty acid composition of cocoa butter from different origins in g/100g (Klagge and Sen Gupta 1990) with abbreviation and carbon number

fatty acid			Ecuador	Brazil	Ghana	Ivory Coast	Malaysia	Java
palmitic	P	16:0	25.6	25.1	25.3	25.8	24.9	24.1
stearic	S	18:0	36.0	33.3	37.6	36.9	37.4	37.3
oleic	O	18:1	34.6	36.5	32.7	32.9	33.5	34.3
linoleic	L	18:2	2.6	3.5	2.8	2.8	2.6	2.7
α-linolenic	Li	18:3	0.1	0.2	0.2	0.2	0.2	0.2
arachidic	A	20:0	1.0	1.2	1.2	1.2	1.2	1.2
sum			99.9	99.8	99.8	99.8	99.8	99.8

are 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1,3-palmitoyl-stearoyl-2-oleoylglycerol (POS) and 1,3-distearoyl-2-oleoylglycerol (SOS) as can be seen in tab. 2.2.

Table 2.2: Main TAG and diglycerides (DG) and sum of C-atoms in the FA chains within the TAG of cocoa butter from different origins in g/100g

TAG		Southeast Asia [1]	West Africa [1]	Central America [1]	Bahia [1]	Ivory Coast [2]
	DG	1.3	0.8	1.4	1.2	
C50	POP	15.0	16.1	17.5	14.7	14.8
	PLP	1.7	1.8	1.7	1.9	
C52	POS	37.4	36.2	37.8	34.2	45.4
	POO	1.9	3.2	2.6	6.2	
	PLS	3.0	3.4	3.1	3.5	
C54	SOS	31.2	27.6	27.3	23.6	28.8
	SOO	3.1	4.9	3.6	8.5	
	SLS	2.2	2.2	2.0	2.0	
	OOO	0.3	0.8	0.5	1.3	
C56	SOA	1.6	1.4	1.3	0.8	
sum		98.7	98.4	98.8	97.9	89.1

[1] Ziegleder, Geier-Greguska, et al. 1994
[2] Dimick and Manning 1987

2.1.2 Milk fat

Beside cocoa butter, milk or butterfat is the only fat in chocolate, which is allowed to be used in amounts higher than 5 %(w/w) in the European Union (European Parliament and Council 2000). Milk fat contains a lot of short-chained FA, as shown in tab. 2.3 for

anhydrous milk fat (AMF), which is made from fresh cream or butter. The minimum fat content of AMF is 99.8 % (Early 2012). The main FA are palmitic, oleic, myristic and stearic acid. FA composition is influenced by season and feeding (Lock and Garnsworthy 2003).

Table 2.3: Fatty acid composition of anhydrous milk fat (AMF) in g/100g

		AMF[1]	AMF[2]	summer AMF[3]	winter AMF[3]	summer AMF[4]	winter AMF[4]
butyric	4:0	4.1		4.9	3.8	5.1	2.7
caproic	6:0	2.4	1.6	2.2	2.3	2.8	0.7
caprylic	8:0	1.3	1.2	1.4	1.5	1.6	0.4
capric	10:0	2.7	2.6	2.5	3.0	3.2	1.4
lauric	12:0	3.2	3.3	3.2	3.7	3.4	2.4
myristic	14:0	10.5	11.5	17.9	11.4	11.2	10.4
myristoleic	14:1			1.6	1.2	0.3	0.4
palmitic	16:0	27.6	31.2	30.5	31.4	29.4	34.7
palmitoleic	16:1			3.2	1.8	1.4	1.5
stearic	18:0	10.6	11.4	11.1	14.1	12.6	16.4
oleic	18:1	25.5	19.9	21.6	25.7	25.3	24.7
linoleic	18:2	2.0	1.1			3.2	3.5
α-linolenic	18:3	0.9	1.0			0.6	0.7
sum		90.8	84.8	100.1	99.9	100.1	89.9

[1] Breitschuh and Windhab 1998
[2] Sabariah et al. 1998
[3] Tietz and Hartel 2000
[4] Williams et al. 1997

Due to the high number of different FA in milk fat, more than thousand different TAGs can be found there. Therefore, the TAGs in tab. 2.4 are only classified by their number of carbon atoms.
However, milk fat is not only used in chocolate itself but also in chocolate fillings. Additionally, high or low melting milk fat fractions are of interest for chocolates or confectionery (Büning-Pfaue and Bartsch 1989; Ziegleder 1993a; Ziegleder 1993b).

2.1.3 Nut and drupe oils

Other fats and oils found in chocolate fillings originate from nuts and drupes, such as hazelnut, almond, pistachio, peanut and macadamia. Their FA composition is shown in tab. 2.5.

The FA and thus the resulting TAG composition varies. This is mainly caused by the origin and whether the oil samples are obtained from raw or roasted material (Crews et al. 2005). Additionally the oil extraction method may have an influence (Bernardo-

Table 2.4: Main TAG according to the number of carbon atoms in the FA chains of anhydrous milk fat (AMF) in g/100g

carbon number sum of FA	winter AMF[1]	high melting fraction[1]	low melting fraction[1]	summer AMF[2]	winter AMF[2]	summer AMF[3]	winter AMF[3]
C32	2.5	1.4	2.9	2.2	2.2	3.1	2.1
C34	4.5	3.2	6.2	5.0	4.6	6.5	5.6
C36	9.4	5.9	11.1	9.9	9.2	11.7	10.8
C38	13.3	9.5	14.5	12.7	12.3	13.8	13.4
C40	11.0	6.6	12.7	9.8	7.9	10.6	10.7
C42	7.0	5.6	6.8	6.4	6.2	6.6	6.6
C44	6.2	6.8	5.3	5.7	5.7	5.8	6.2
C46	6.8	10.1	5.4	6.4	6.5	6.4	6.7
C48	8.6	14.0	6.5	8.4	8.7	7.8	8.7
C50	11.5	18.3	8.7	11.8	12.4	9.7	11.1
C52	11.5	12.1	10.8	11.8	12.5	8.6	10.7
C54	5.4	5.8	6.7	5.5	5.8	4.9	5.6
sum	97.7	99.3	97.6	95.6	94.0	95.5	98.2

[1] Herrera et al. 1999
[2] Tietz and Hartel 2000
[3] Williams et al. 1997

Table 2.5: Fatty acid composition of different nut and drupe oils in g/100g (rounded mean values from Krist and Biladt 2013)

	palmitic (P) 16:0	stearic (S) 18:0	oleic (O) 18:1	linoleic (L) 18:2	α-linolenic (Ln) 18:3	sum
hazelnut	6	2	83	9	traces	100
almond	5	2	70	20	0	97
olive	13	2	67	12	traces	94
pistachio	9	2	65	20	traces	96
canola	4	0	60	22	9	95
macadamia	9	3	60	2	0	74
peanut	10	3	50	35	traces	98
sunflower	7	5	27	61	traces	100
linseed	5	4	20	18	53	100
safflower	4	2	19	71	1	97
walnut	5	2	17	62	10	96
hampseed	6	2	11	60	21	100

Gil et al. 2002). Macadamia oil is the only oil which also includes high amounts of palmitoleic acid of about 20 %(w/w). The FA composition of all oils and fats depends on the origin. This is especially reported for hazelnut, olive and walnut oil. Furthermore different varieties exist. In the case of sunflower oil, there are several varieties with an increased amount of one or two fatty acids, such as high-linoleic or high-oleic sunflower oil (Krist 2013b). Due to the high amount of oleic and linoleic acid found in all nut and drupe oils, the main TAG are OOO, LOO, LLO and LLL, as can be seen in tab. 2.6.
The TAG composition of hazelnut oil depends on genotype, origin, climate, maturity and malibration. Thus, hazelnuts of increasing diameter show a significant increase in OOO content while LLL, LLO and LOO contents decrease (Rieblinger and Ziegleder 1995).

Table 2.6: Main TAG and sum of carbon atoms in the FA chains within the TAG of different nut and drupe oils in g/100g (Krist and Biladt 2013)

		hazelnut	almond	peanut	linseed	olive	sunflower
C48	PPL	traces		2	14	1	1
C50	PPO	traces	traces	1		4	traces
C52	POO	10	9	6	4	22	2
	PLL	traces	3	7	11	1	9
	POL	2	9	13	14	8	6
C54	SOO	4	3	3		4	1
	OOO	67	31	10	2	30	3
	OOL	14	25	20	4	10	12
	OLL	2	16	20	7	1	26
	LLL	1	3	4	4	traces	21
sum		100	99	85	60	77	81

P: palmitic; S: stearic; O:oleic; L:linoleic acid

2.2 Crystallization of vegetable fats and oils

Crystallization is the transfer of a material from an amorphous, liquid or gaseous into a crystalline state. Crystals are solids whose constituents, such as atoms or molecules form a highly ordered structure which extends in all directions. The smallest unit is called elementary cell, whose periodic, three dimensional continuation results in the crystal lattice. Crystallizing from a melt includes nucleation and crystal growth. Depending on the growth rate, crystal growth can be divided in main crystallization followed by post crystallization. The three phases of crystallization are shown in fig. 2.4.

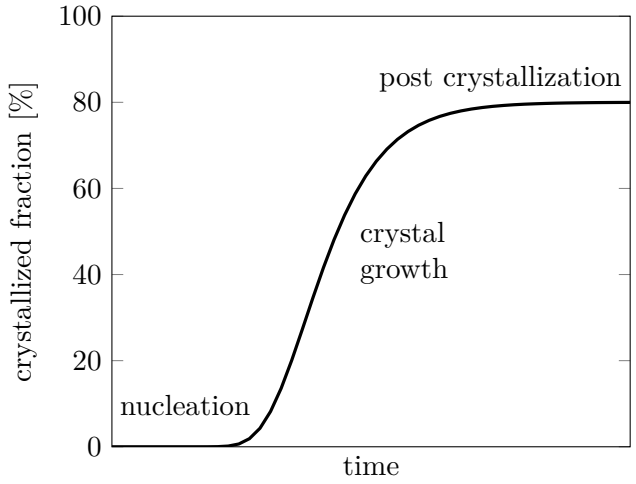


Figure 2.4: Three phases of crystallization

In the first phase, the nucleation, stable and viable nuclei have to be formed. Therefore, the Gibbs free energy, which indicates whether a reaction is spontaneous ($\Delta G < 0$) or not ($\Delta G > 0$) has to be taken into account. The Gibbs free energy ΔG depends on the changes of entropy ΔS and of enthalpy ΔH together with temperature T, as shown in eq. 2.2.1.

$$\Delta G = \Delta H - T \cdot \Delta S \tag{2.2.1}$$

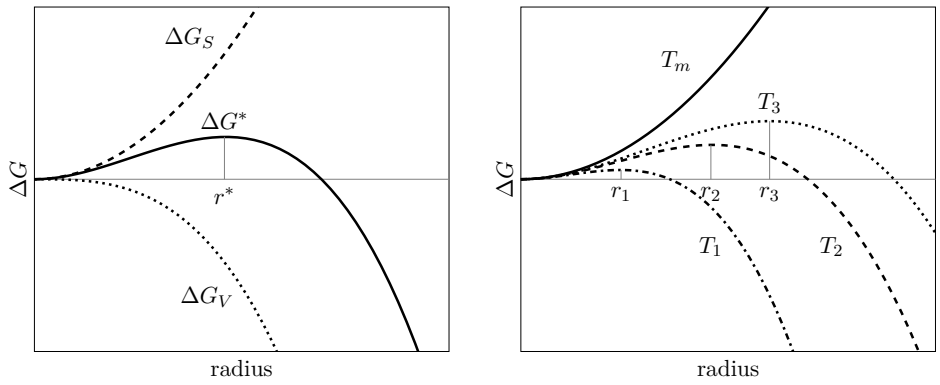
According to Ziegleder 1995, the Gibbs free energy of crystallization can also be described as the sum of the energy which is released during crystallization of the supercooled volume and the energy needed to build up the surface of crystals, as can be seen in eq. 2.2.3.

$$\Delta G = A \cdot \Delta G_A - V \cdot \Delta G_V \tag{2.2.2}$$

$$\Delta G = 4\pi r^2 \cdot \sigma - \frac{4}{3}\pi r^3 \cdot \Delta H \frac{T_m - T}{T_m \cdot V_m} \tag{2.2.3}$$

ΔG is the Gibbs free energy, ΔG_V is the volume related energy and ΔG_A the sur-

face/area related energy, with r being the crystal nuclei radius. T is the temperature and index m indicates melting with ΔH being the melting enthalpy and σ is the surface tension. The supercooling, which induces crystallization is expressed by the term $\Delta T = T_m - T$.



(a) Dependency of nuclei radius (b) Different temperatures

Figure 2.5: Gibbs free energy ΔG (a) in dependency of nuclei radius with critical radius $r*$ at maximum ΔG with ΔG_V as volume related energy and ΔG_S as surface related energy and (b) for different temperatures $T_1 < T_2 < T_3$ below melting temperature T_m and according critical nuclei radius $r_1 < r_2 < r_3$

Fig. 2.5a illustrates the relation of the parameters. The parameters ΔG^* and r^* are the critical Gibbs free energy and critical crystal nuclei radius, above which the nuclei are stable and start to grow (Gibbs 1874-78). The temperature dependency of nucleation is shown in fig. 2.5b. It has to be noted that too high supercooling (too low temperatures) lead to a reduced mass transport and decreased diffusivity (Toro-Vazquez et al. 2000). However, the supercooling used to induce crystallization in fat systems is generally low, only a few degrees (Walstra et al. 2001).
Thermal crystallization at different supercooling temperatures leads to different critical radii. Lower supercooling temperatures, e.g. $\Delta T = T_m - T_3$ result in less and bigger nuclei than high supercooling temperatures such as $\Delta T = T_m - T_1$. Intensified supercooling also leads to higher nucleation rates (Himawan et al. 2006).
Athermal crystallization, during which temperature is continuously reduced causes a change of nuclei stability. Nuclei with same radius r_2 are instable at temperatures higher than T_2 but become stable when temperature is reduced below T_2.
During nucleation crystal nuclei are formed in a homogeneous or heterogeneous way (Dimick 1999). Homogeneous nucleation occurs in melts without surfaces (i.e. particle surfaces) that serve as activators for nuclei formation (Avrami 1939). This kind of nucleation is also called primary nucleation. If surfaces are present, the nucleation is called heterogeneous (Dimick 1999). Heterogeneous nucleation needs less change of free enthalpy than homogeneous nucleation and therefore less supercooling. The reason is supposed to be a local arrangement caused by interaction at the surface (Dimick 1999;

Himawan et al. 2006; Garside 1987). A special kind of heterogeneous nucleation is secondary nucleation. Primary crystals are broken up, e.g. by shear forces, into secondary crystals which can act as new nuclei or are intentionally added in the course of the process.
After the critical cluster size r^* has been exceeded, crystal growth starts (Himawan et al. 2006). The crystal growth rate depends on the number of nuclei, their surface and structure as well as the temperature (Cebula and K. W. Smith 1991; Himawan et al. 2006). Crystal growth is mainly depending on the crystallizing substances. During post-crystallization the crystal surfaces are restructured and the crystallization degree increases further (Dimick 1999). Crystal growth and post-crystallization highly depend on the crystallizing material and will be further described for TAG in the next chapter.

2.2.1 Crystallization of TAG

TAG, which are the main component of edible fats and oils, often show polymorphic behavior, which means that more than one crystal form exists for a chemical identical melt. A distinction is drawn between enantiotropic and monotropic polymorphism. Enantioropic polymorphism shows thermodynamically stable polymorphic forms in a particular temperature and pressure range which are reversible and convertible into each other. In monotropic polymorphism, the transition proceeds in only one direction and the polymorphic forms exhibit different thermodynamic stability. Polymorphism is a matter of special importance for TAG, especially those with high amounts of symmetric, monounsaturated FAs (Afoakwa, Paterson, M. Fowler, and Vieira 2009b; Talbot 2009b; Hubbes, Danzl, et al. 2018).
An overview of fat crystallization is given by Himawan et al. 2006; Sato 2001; Sato et al. 1999. However, a lot of research is done on pure TAG systems, while real fats and oils are mixtures of different TAG. These TAG include saturated and unsaturated fatty acids, where the unsaturated fatty acid is in the middle position of the glycerol backbone. Therefore they are called TAG of the 'sat-O-sat' type (sat: saturated; O: oleic acid - unsaturated fatty acid). Due to the kink of the unsaturated oleic acid at the sn-2 position, specific structures have to be formed. Compared to saturated TAG structures, an intermediate γ phase can be found and a triple chain-length structure is formed. This structure is representative for most TAG of the sat-O-sat type, except POP, which shows a double chain-length structure in β' crystal polymorph, as can be seen in fig. 2.6 (Yano et al. 1993).

The crystal structure and phase behavior of binary and ternary mixtures of POP, POS and SOS have been investigated by several authors (K. W. Smith, Bhaggan, and Talbot 2013; Tran et al. 2015; Miura et al. 2004; Rousset et al. 1998). Especially these ternary mixtures are used to describe the phase behavior of natural fats (K. W. Smith, Bhaggan, and Talbot 2013; Hubbes, Danzl, et al. 2018).
Cocoa butter, which includes 0.75 g/g to 0.85 g/g POP, POS and SOS, shows monotropic polymorphism with up to six forms: the unstable γ and α forms, which melt below 20 °C; the metastable β' forms, which are either interpreted as a range of β' forms or as two forms β'_{III} and β'_{IV} , and the most stable forms β_{V} and β_{VI} , which are of interest for

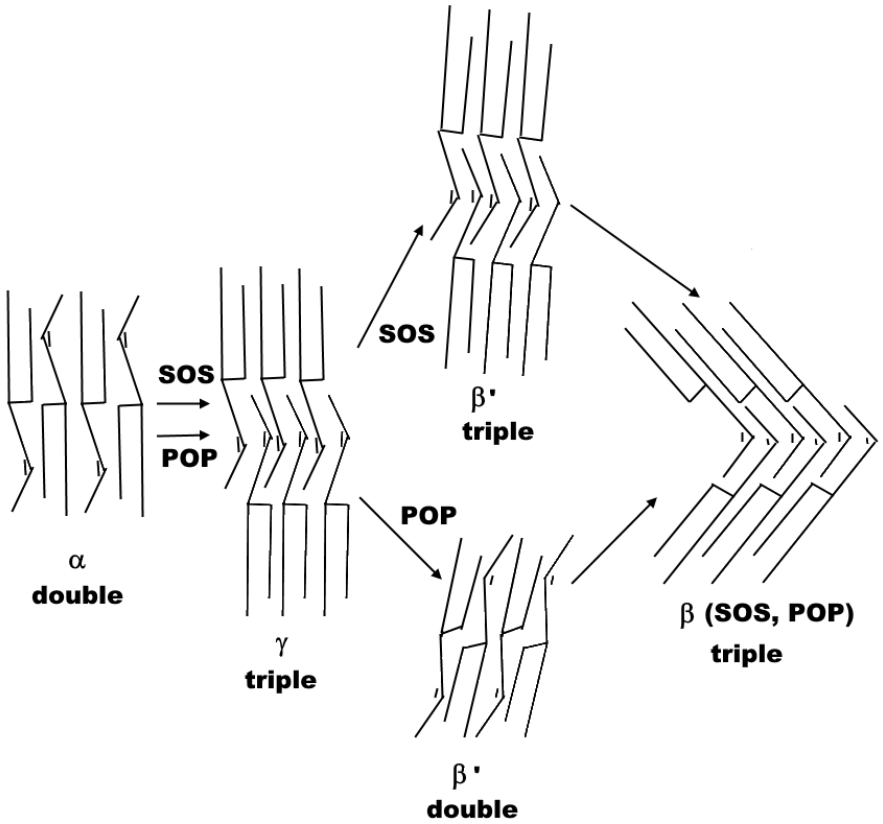


Figure 2.6: Structure model of the polymorphic forms of POP and SOS (modified after Yano et al. (1993))

the manufacturing industry (van Malssen, Langevelde, et al. 1999; Wille and Lutton 1966; Timms 1984). The nomenclature and melting temperatures are listed in tab. 2.7.

Table 2.7: Polymorphic forms of cocoa butter according to different authors and their nomenclatures

Nomenclature			Melting temperature [°C]				chain
[1, 2]	[3]	[4]	T_{melt} [1]	T_{melt} [2]	T_{melt} [4]		length [5]
I	sub-α/ γ	γ	17,3	13,0	-8,0	5,0	double
II	α	α	23,3	20,0	17,0	22,5	double
III	β'	β'_{III}	25,5	23,0	19,6	29,0	double
IV	β'	β'_{IV}	27,5	25,0			double
V	β	β_V	33,8	30,0	28,0	33,7	triple
VI	β	β_{VI}	36,3	33,5			triple

[1] Wille and Lutton 1966
[2] Lovegren, Gray, et al. 1976
[3] Vaeck 1960
[4] van Malssen, Langevelde, et al. 1999
[5] Talbot 2009b

As a matter of practical applications, not only pure TAG but also other fats and oils are mixed and analyzed regarding their crystallization and phase behavior. The phase behavior of the mixtures depends on the type of fat or oil which is added. Fats with symmetrical TAGs, such as POP, POS and SOS are compatible (Gordon et al. 1979). Cocoa butter seed crystals contain higher levels of SOS in comparison to cocoa butter (Dimick and Manning 1987; Schlichter-Aronhime, Sarig, et al. 1988; van Malssen, Peschar, et al. 1996). Some authors reported an increased level of tristearin (SSS) in cocoa butter seed crystals. Bloom on dark chocolate showed relatively higher levels of POP (Ziegleder, Geier-Greguska, et al. 1994).
In general, cocoa butter crystals may incorporate 0.05 g/g to 0.1 g/g of liquid TAG into the crystal lattice (Rothkopf and Danzl 2015). However, common additions to cocoa butter are milk fat and hazelnut oil as model samples to gain knowledge about milk chocolate and filled products. Lovegren, Gray, et al. 1976; K. W. Smith, Cain, et al. 2007 found that hazelnut and olive oil addition to cocoa butter accelerated polymorphic transition and in the case of olive oil fewer polymorphic forms were observed (Lovegren, Gray, et al. 1976).
Vegetable oils mainly include oleic and linoleic acid and therefore consist of few TAG, namely triolein (OOO), trilinolein (LLL), 1-linoleoyl-2,3-dioleoylglycerol (LOO) and 1,2-dilinoleoyl-3-oleoylglycerol (LLO). Especially OOO is known to be incorporated into the cocoa butter lattice easily up to amounts of 0.12 g/g (Ziegleder, Geier-Greguska, et al. 1994). Milk fat includes up to 400 fatty acids, which leads to a theoretical number of 400^3 possible TAG (Breitschuh and Windhab 1998). This great variety leads to a broad melting temperature range and complex physicochemical properties (Metin and Hartel 1996). The usage of milk fat and milk fat fractions in chocolate and its effect on crys-

tallization has been investigated for decades: Hogenbirk 1990; Metin and Hartel 1996; Metin and Hartel 1998; Pajin et al. 2012; Petersson 1986; Schmelzer and Hartel 2001; Sonwai and Rousseau 2010; Tietz and Hartel 2000; Timms 1984.
Minor components, such as fatty acids, mono- and diglycerides as well as emulsifiers have an influence on crystallization. The main emulsifier used in chocolate is lecithin, which reduces the viscosity of the chocolate to facilitate chocolate manufacturing (Minifie 1989). Lecithin is a natural product and includes a mixture of phospholipids, TAG, FFA and carbohydrates. Its impact on crystallization depends on the type of lecithin, the amount used and the combination with other ingredients (Kindlein et al. 2015; Ribeiro et al. 2015). The size of the spherulites was reduced in static crystallized pure cocoa butter when lecithin was added (Bowser 2006). The impact of lecithin on pure cocoa butter crystallization may be caused by solids in the lecithin which act as crystal nuclei. During crystal growth, the so-called button-syndrome can occur (Schlichter-Aronhime and Garti 1988). The button-syndrome describes the incorporation of emulsifiers in the crystal lattice. The incorporated emulsifier reduces the rate and amount of polymorphic transition into the stable β-form due to its particular chemical and structural fit (Schlichter-Aronhime and Garti 1988).
In mixtures of cocoa butter with sugar and lecithin the crystallization is accelerated (Svanberg et al. 2011b). The interaction of sugar particle surfaces and the TAG is probably increased by the emulsifier. Under shearing conditions a crystallization delay was observed by Dhonsi and Stapley 2006, which was explained by a reduced effective shear input caused by the emulsifier.
As was already mentioned, lecithin is used in chocolate , where also cocoa and sugar particles are present. They cause heterogeneous nucleation, so that the impact of lecithin on nucleation might be neglected (Dimick 1999). To verify this, Bowser 2006 investigated the impact of cocoa and sugar particles on cocoa butter crystallization with and without lecithin addition. It was found that in isothermal nuclear magnetic resonance (NMR) measurements cocoa particles shortened the induction time, while sugar particles showed no effect without lecithin addition. In samples with lecithin, a slight shortening effect of sugar particles on induction time was found. The results were confirmed by Svanberg et al. 2011b for seeded cocoa butter. Svanberg et al. 2011b found that 5 mg/g lecithin had an significant impact on crystal growth in seeded cocoa-butter-sugar-suspensions, since the amount of stable crystals was increased as measured by differential scanning calorimetry (DSC) melting curves. Further studies with emulsifiers including acyl groups similar to those of fat showed an overall faster crystallization (Ribeiro et al. 2015). An increased crystal growth rate allows more time for instable polymorphs to transform to more stable ones (Bowser 2006; Svanberg et al. 2011b).
Cocoa-butter-cocoa-powder-suspensions showed a higher crystal growth rate than cocoa-butter-sugar-suspensions. The morphology of the crystals was influenced by cocoa powder addition, which was probably caused by the residual fat content of 0.10 g/g to 0.12 g/g, according to Svanberg et al. 2011b.
The cocoa particles themselves can act as nuclei and might accelerate nucleation. Heterogeneous nucleation on surfaces is affected by the interface energy of the fluid-crystal

interface, fluid surface and crystal surface (Garside 1987). Thus the lipophilic TAG are more likely to be immobilized on the lipophilic cocoa particle surface than on the lipophob sugar surface (Svanberg et al. 2011b).

2.2.2 Polycrystalline structure and microstructure

The polycrystalline structure is responsible for the microstructure and resulting mechanical properties of pure fats (Hubbes, Braun, et al. 2020a). The influencing factors for the microstructure are crystal amount, size, morphology and polymorphism. Since these factors depend on crystallization time and temperature, the processing conditions have an impact on the microstructure of crystallized fat.
The interaction of crystallization behavior, microstructure and mechanical properties was studied by Brunello et al. 2003. They found that, besides solid fat content (SFC), polymorphism strongly influences the microstructure. Statically crystallized cocoa butter showed granular morphologies, large spheres, clusters of small spheres or a continuous microstructure depending on crystallization temperature and time.
The polymorphism of cocoa butter strongly depends on pre-crystallization, also named tempering. In case of cocoa butter replacer (CBR), cooling rate influenced mechanical properties, such as storage modulus and hardness in stearic rich fats. In palmitic rich fats brittleness was affected to a higher extent than hardness and storage modulus (Gregersen et al. 2015; Hubbes, Braun, et al. 2020b). In AMF and lard crystallization, the fat crystal network also depends on the cooling rate (Campos et al. 2002). The impact of polymorphism becomes visible when density is regarded. A higher density could be observed with increased stable polymorphic forms for POP, POS and SOS. The increase in density leads to contraction during crystallization which facilitates demolding. Also cracks and crevices might be formed (Arishima, Sagi, et al. 1995). The microstructure of fats with suspended particles will be described in chap. 2.3.1.

2.2.3 Measuring rate and degree of cocoa butter crystallization

Besides TAG composition, there are other factors influencing crystallization and polymorphism, such as temperature, including cooling and heating rate, pressure, solubility and impurities (Sato 2001). These factors can be varied within different measuring apparatuses and conditions.
There are two types of crystallization in fats and oils: solid-solid transition, which describes the polymorphic transition, and the solidification from liquid to solid. For the latter the sample has to be initially in a molten state. The crystallization is induced by supercooling. Therefore the sample can be cooled rapidly to a temperature and held there isothermally for some time. Another possibility is the use of a constant cooling rate. Typical apparatuses for measuring crystallization in fats and oils are DSC, NMR or X-ray diffraction (XRD). XRD measurements are used to gain information about the crystal lattice. DSC detects changes in the samples which are accompanied by phase transitions and NMR can be used to investigate the electronic structure of single atoms and their interaction with atoms surrounding them.

DSC can be used for measuring under isothermal conditions or using a constant cooling rate (Foubert, Vanrolleghem, and Dewettinck 2003; Ziegleder 1988; Ziegleder 1985; Fessas et al. 2005). However, for DSC measurements, the sample state has to be changed. Therefore it is a destructive measuring technique. In contrast NMR is a nondestructive measuring technique. For example measuring the SFC is a well-established method described in DIN EN ISO 2010a; DIN EN ISO 2010b, which are based on Petersson et al. 1985; Petersson 1986. However, NMR can also be used to measure crystallization kinetics by measuring the same sample frequently during cooling (Padar, Jeelani, et al. 2008; Bootello et al. 2013; Bowser 2006). Due to the big sample volume used for NMR measurements the heat of crystallization causes warming of the sample. Therefore crystallization kinetics measurements using NMR should rather be named quasi-isothermal. The release of crystallization heat is used for Shukoff measurements and the Bühler MultiTherm™ TC, which is based on the Shukoff principle (Shukoff 1899; Shukoff and Schtschawinsky 1903). The completely molten sample is placed in a glass flask (Shukoff) or an aluminum cup (MulitTherm™) which is then placed in a water bath. The temperature of the sample is recorded over time and the resulting cooling curve can be evaluated. Other methods used to measure fat crystallization are turbidimetry or polarized light microscopy. However, these methods can solely be used for detecting the crystallization start due to saturation after a short time (Marangoni 1998; Bowser 2006). Viscosity and ultra sonic shear reflection measurements are also possible (Rigolle et al. 2015; Foubert, Dewettinck, and Vanrolleghem 2003). For all methods which are suitable to describe the crystallization change over time a typical sigmoidal curve progress, as shown in fig. 2.4, can be obtained.

2.3 Chocolate as a dispersion

A dispersion consists of two or more in-miscible substances. One or more substances are dispersed in a continuous phase. Dispersions can be divided in groups according to the state of matter of the individual phases (Lagaly et al. 1997). For instance, solids dispersed in liquids are called dispersions, while solids in air are called aerosols. Dispersions can be further divided according to the particle size of the dispersed phase. Liquids with dispersed solids with a size of less then 1 nm are called solutions, with particles from 1 nm to 1 µm, they are called sol and in the case of bigger particles with diameter of more than 1 µm, they are called suspensions (Bahadir et al. 2000).
In the case of molten chocolate, solid sugar, cocoa and optionally milk particles are dispersed in a continuous liquid fat phase. The size of particles present in chocolate is 4 µm to 30 µm, which is bigger than 1 µm thus, chocolate is a suspension. The technically useful particle size is limited by a sandy mouth feel of too big particles. Too small particles have a big surface compared to their volume, where fat can be bound, which increases the needed cocoa butter amount. The particle size is not uniform instead it covers a broad range due to processing and mechanical properties of the particles.
Due to the non-uniform dispersity of particle size in chocolate, small particles are able to fill the interspace between big particles, as is schematically shown in fig. 2.7. In crystallized chocolate the fat is only partly solid, thus the continuous liquid oil phase is decreased while the dispersed phase is increased compared to liquid chocolate.

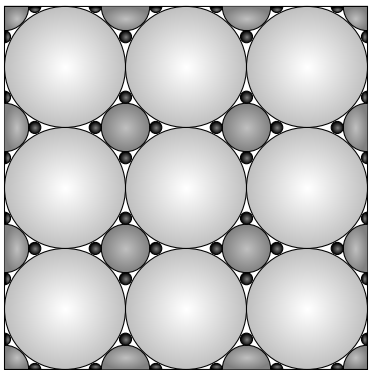


Figure 2.7: Schematic two dimensional drawing of a trimodal particle size distribution with big (lightgray), medium (gray) and small (black) round particles

However, the filling of particle interspace by smaller particles is also limited due to an enlarged surface. Therefore the particle size in chocolate is limited on the small size because of the adsorption of fat to the particle surface (Beckett 2009a). The particle volume is given by the chocolate recipe, while the surface depends on the particles size. The surface (S) to volume (V) ratio is reciprocally proportional to the radius, which can be seen in fig. 2.8.

These theoretical calculations have to be adjusted since particles in chocolate are ir-

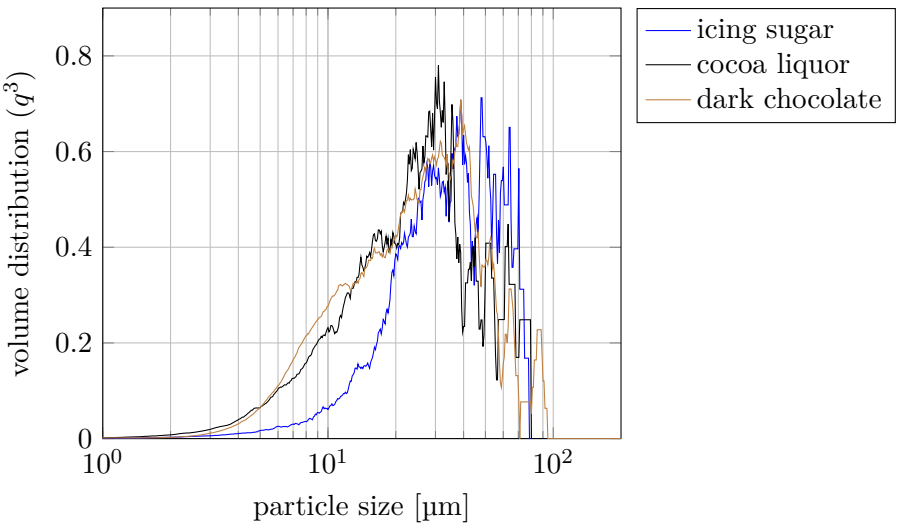


Figure 2.8: Surface S to volume V ratio in dependency of particle radius or edge length r for spheres and cubes

regular nuggets and plates instead of spheres. Spheres show the minimum surface to volume ratio resulting in an higher ratio for all other shapes, such as cubes.
As already mentioned, the continuous phase has to be available as a flux to allow flowing of the chocolate mass (Do et al. 2011). Regarding chocolate, the particles are agglomerated after roll refining and the fat is enclosed in these agglomerates. Additionally the particle surfaces are not fully covered with fat (Ziegleder, Balimann, et al. 2003). During conching the agglomerates are broken down, the particle surfaces are covered with fat and some trapped fat might be pressed out of the cocoa particles due to the mechanical energy input. This could be shown by a drastic decrease in pore volume, as can be seen in fig. 2.9, and increase of fat removable by centrifuging, with increased dry conching time (Ziegleder, Balimann, et al. 2003).

The impact of trapped fat on flow properties of chocolate was intensively studied for milk powder (Attaie et al. 2003; Haylock and Dodds 2009; Liang and Hartel 2004). Do et al. 2011 studied the flow properties of chocolate prepared with cocoa mass, standard defatted cocoa powder and highly defatted cocoa powder. Their results were ambiguous due to different particle size and shape. However, they found that cocoa particles show a porous structure with trapped fat on the pores surface, as can be seen in fig. 2.10.

The specific surface of chocolate with different particle sizes and fat content was calculated from particle size distribution by Afoakwa, Paterson, and M. Fowler 2008 and ranged from $1\,m^2/g$ to $2\,m^2/g$ depending on particle size and fat content. The specific surface area and surface polarity of icing sugar and cocoa was also determined by Franke et al. 2011, where the specific surface was $0.27\,m^2/g$ for cocoa powder and $0.55\,m^2/g$ for sugar. For the surface energy the ratio of dispersive to polar was 4.50 for cocoa powder and 3.68 for the sugar (Franke et al. 2011). However, particle size is not explicitly given;

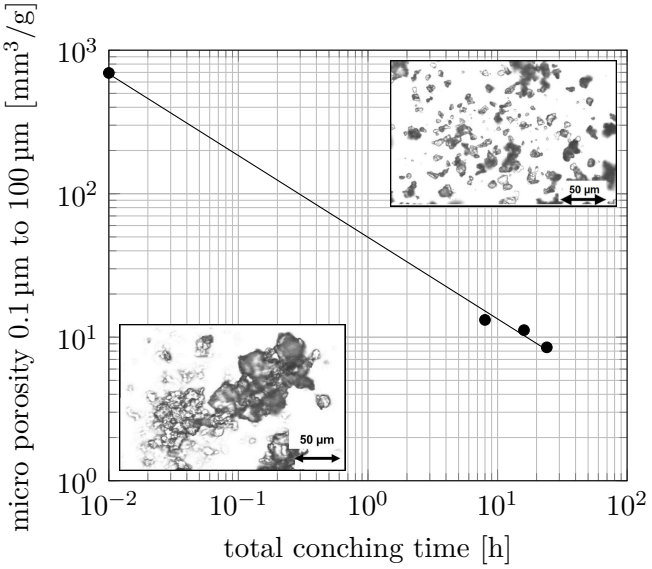


Figure 2.9: Decreasing micro porosity with increasing conching time as shown by Ziegleder, Balimann, et al. 2003

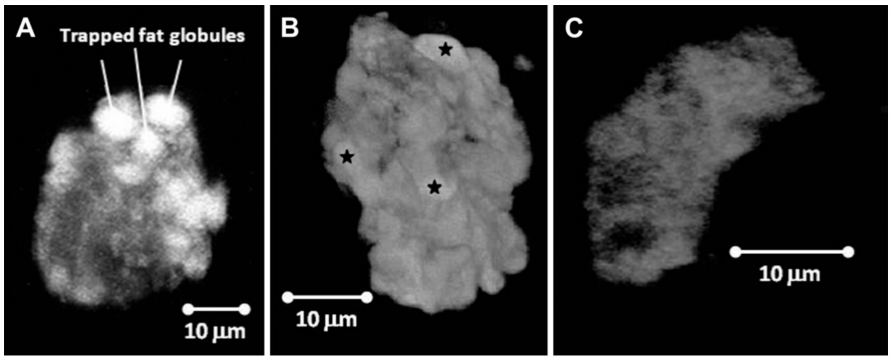


Figure 2.10: confocal laser scanning microscopy (CLSM) micrographs of cocoa particles. A: cocoa mass; B: cocoa powder with 11 g/100g fat; C: highly defatted cocoa powder with < 1 g/100g fat (Do et al. 2011)

the sugar was sieved to be below 90 µm and no details are given for the cocoa powder concerning particle size and fat content.

2.3.1 Microstructure of solidified chocolate

The microstructure of chocolate was studied with focus on mechanical properties and impact on migration mechanism by several authors (Dahlenborg 2014; Dahlenborg, Millqvist-Fureby, et al. 2015a; Lee et al. 2010; Maleky et al. 2012; Miquel et al. 2001; Motwani et al. 2011; Svanberg et al. 2013; Svanberg et al. 2011a; Svanberg et al. 2011b). According to Delbaere et al. 2016, four influencing factors in terms of chocolate microstructure are composition, processing conditions, post-processing and storage conditions. The amount of solids in solidified chocolate is higher than in liquid chocolate due to the partly crystallized cocoa butter. The crystallization process of cocoa butter and the impact of particles on chocolate flow properties were described in chap. 2.2.2. Both have an impact on final chocolate structure, which is characterized by cocoa particles, sugar crystals, solidified cocoa butter and, in case of milk chocolate, milk powder in a continuous phase of liquid cocoa butter (Aguilera et al. 2004; Afoakwa 2010). In chocolate two effects overlay: the solids interact with each other and the solids might affect crystallization.
The particle size in chocolate is limited by mouth feel and flow properties. However, chocolates with different particles sizes and particles size distributions are available. Afoakwa investigated a broad range of parameters, influenced by particle size and distribution. Chocolates made with smaller particles, as well as chocolates with lower fat content showed higher hardness, probably caused by particle interaction (Afoakwa, Paterson, M. Fowler, and Vieira 2009c). Particle interactions can be caused by van der Waals forces and hydrogen bonds in the case of polar particles. Hence particle networks can be formed (Delbaere et al. 2016). In addition, the interaction of particles and fat influences the microstructure.
The main particle component is sugar, mainly sucrose or additional lactose in milk chocolates. Both sugars can be present in amorphous or crystalline state, which has an impact on microstructure (Bricknell and Hartel 1998). The microstructure is mainly influenced by the shape of the sugar particles. While amorphous sugars are round, crystalline ones show sharp edges (Bricknell and Hartel 1998). The different surface properties also have an impact on microstructure (Delbaere et al. 2016). The surface properties of the particles can be changed by lecithin addition, as mentioned in chap. 2.2, which affects crystallization properties. Svanberg et al. 2011b compared the crystallization properties of cocoa butter with sugar or cocoa powder or a mixture of both. They found that cocoa particle addition accelerated nucleation and crystal growth compared to sugar addition. The difference in crystallization properties could be reduced by lecithin addition, which mainly had an impact on cocoa-butter-sugar-suspensions (Svanberg et al. 2011b). With regards to microstructure cocoa powder addition resulted in an inhomogeneous visual appearance of the crystal structure of the cocoa butter with more stable crystals. Samples seeded with cocoa butter crystals showed a very homogeneous visual appearance and dense structure (Svanberg et al. 2011a). Thus the inhomogeneous crystal struc-

ture caused by cocoa powder addition could be reduced by proper pre-crystallization (Svanberg et al. 2011b). However, tempering under shear, which will be described in chap. 2.5.2, leads to different structures than tempering by seeding (Svanberg et al. 2013; Svanberg et al. 2011b). Shear tempering is affected by particles, especially by particle size distribution and amount. This is due to the changed rheological behavior where the realizing of shear is affected and the effective heat capacity, which is different for fat, cocoa and sugar particles (Afoakwa, Paterson, M. Fowler, and Vieira 2008c; Rothkopf and Danzl 2015). Nevertheless, particle size distribution had no effect on crystallinity of chocolate, independent of tempering regime, which was shown by investigating the melting behavior (Afoakwa, Paterson, M. Fowler, and Vieira 2009b; Afoakwa, Paterson, M. Fowler, and Vieira 2008a). Beside melting behavior, the hardness was also measured. In this case samples containing small particles with dimensions less than 18 µm showed a higher hardness for all tempering regimes (Afoakwa, Paterson, M. Fowler, and Vieira 2008b). Comparison of tempering regimes showed increasing hardness from optimal to over- and under-tempered (bloomed) samples (Afoakwa, Paterson, M. Fowler, and Vieira 2008b).

As already mentioned, particles influence the flow behavior of the chocolate melt. They cause a yield stress, i.e. a force that must be overcome to make the chocolate flow. Therefore liquid chocolate has to be vibrated to remove air bubbles, which have to less uplift to overcome the yield stress. These air bubbles could result in macro pores after solidification if they are not removed. However, air could also be dissolved in the chocolate and cause micro pores. Using mercury porosimetry, it could be shown that solidified chocolate has a porous structure (Loisel et al. 1997). In well-tempered chocolate with 319 mg/g cocoa butter cavities occupied 10 cm^3/m^3 of the volume. A decrease of cocoa butter amount to 295 mg/g increased the pore volume to about 20 cm^3/m^3. While under-tempering (too less pre-crystallization) had no effect, over-tempered samples (too much pre-crystallization) showed an increased pore volume of about 40 cm^3/m^3. Reinke, Wilde, et al. 2015 confirmed the presence of cracks and crevices especially near air bubbles and particles using synchrotron X-ray microtomography. They stated that the cracks are formed due to local stress during cooling caused by different contraction of cocoa butter and particles (Reinke, Wilde, et al. 2015).

Beside pores, the crystallinity influences migration and vice versa. While the impact of the solid fat content was already studied (Jin and Hartel 2015), the influence of polymorphism is unknown. However, K. W. Smith, Cain, et al. 2007 showed that migrating hazelnut oil changes the polymorphism by accelerating the β_V to β_{VI} transition.

The microstructure is especially important when it comes to migration from a softer filling into the chocolate. Studies on oil migration and the impact factors were done to investigate fat bloom formation. This will be further described in chap. 2.6. Observing the migration of oil in chocolate with regard to microstructure is challenging, due to the dark color. Magnetic Resonance Imaging (MRI) is a measuring method, where the migration of filling fat into chocolate can be observed over a certain time period, which also allows to determine the spatial distribution at a resolution of 200 µm/pixel (Miquel et al. 2001).

The impact of particle size of the sugar and cocoa particles on oil migration in chocolate is contradictory. On the one hand, bigger particles lead to a more porous structure, which accelerates migration (Choi, K. L. McCarthy, M. J. McCarthy, and Kim 2007). On the other hand, Altimiras et al. 2007; Dahlenborg, Millqvist-Fureby, et al. 2015a found that samples with small particles show faster migration. This might be caused by the increased surface of non-fat solids. The increased surface is accompanied by a more heterogeneous network of non-fat particles and crystallized fat, which leads to higher permeability (Altimiras et al. 2007; Dahlenborg, Millqvist-Fureby, et al. 2015a). In both cases the impact of particles can be explained by a changed fat crystal structure, which is more heterogeneous and has more pores. The pores are big enough to cause capillary flow (Aguilera et al. 2004; Rousseau and P. Smith 2008; Reinke, Roth, et al. 2015). The start of pore formation was also found on the chocolate surface during storage, where they might be important for fat bloom formation (Rousseau and P. Smith 2008; Sonwai and Rousseau 2006).
The addition of particles might change the migration mechanism of oil in chocolate. TAG might migrate through particle interspace (Ghosh et al. 2002; Ziegler, Shetty, et al. 2004; Ziegler and Szlachetka 2005). The way of the migrating oil is enlarged by a higher tortuosity caused by the particles compared to samples without particles (Galdámez et al. 2009; Ghosh et al. 2002; Motwani et al. 2011). Lee et al. 2010; Svanberg et al. 2011a found that the addition of cocoa particles and sugar leads to a reduced migration in tempered cocoa butter. However, Reinke, Roth, et al. 2015; Dahlenborg, Millqvist-Fureby, et al. 2015b; Motwani et al. 2011 observed an increased migration in samples with particle addition. The migration rate in samples of cocoa butter with sugar was lower than in mixtures of cocoa butter with cocoa particles (Dahlenborg, Millqvist-Fureby, et al. 2015b). The impact of particle surface and properties, such as hydrophobicity or specific surface is still unclear. The addition of emulsifiers on migration results is also controversial. Emulsifiers affect crystallization and microstructure, therefore they might also have an impact on migration (Delbaere et al. 2016). Timms 2002 found that lecithin addition increased fat migration, while Bueschelberger 2004 found that the use of deoiled lecithin instead of liquid lecithin showed contrary effects. Thus, all factors influencing microstructure, such as crystallization, particles and surface active agents, also affect migration in chocolate products.

2.4 Migration of filling lipids into chocolate

Migration here describes the movement of a substance from one place to another across a single boundary. This chapter will focus on mass transport of chemical species. Mass transport is a common phenomenon in food, especially during storage. In most cases moisture migration is the cause for quality loss. This might also be the case for chocolates filled with fillings with water as the continuous phase, such as alcoholic/liqueur fillings, fondant, ganaches and butter cream fillings. In this work the focus was on fillings with fat as the continuous phase, such as nougat or marzipane. In chocolates with fat based fillings, fat migration is the main cause for fat bloom formation and texture

changes (Delbaere et al. 2016).
The driving force for migration is the concentration gradient and the transport mechanism is usually related to chocolate properties. However, besides fat composition and SFC, the binding of the liquid oil in the filling might affect migration. Due to the complex microstructure of chocolate, which is further described in chap. 2.3, the migration mechanism is still not fully understood. The first hypotheses by Ziegleder, Moser, et al. 1996a was that diffusion is the transport mechanism for fat migration in chocolate (Ziegleder, Moser, et al. 1996b; Galdámez et al. 2009; Ghosh et al. 2002; Lee et al. 2010; Maleky et al. 2012; K. L. McCarthy and M. J. McCarthy 2008; Motwani et al. 2011). Another hypothesis discussed by Aguilera et al. 2004 mentions capillary flow as transport mechanism. This hypothesis is also described by others (Choi, K. L. McCarthy, and M. J. McCarthy 2005; Guiheneuf et al. 1997). The theory of capillary flow is complemented by the theory of driven convective flow (Altimiras et al. 2007; Dahlenborg, Millqvist-Fureby, et al. 2015a; Loisel et al. 1997). In the last decade a combination of both mechanisms has also been mentioned (Deka et al. 2006; Reinke, Hauf, et al. 2015; Rousseau and P. Smith 2008). Diffusion or capillary flow as transport mechanism of oil from the filling through the fat phase of chocolate will be further described in the following sections chap.2.4.1 and 2.4.2.

2.4.1 Diffusion through the fat phase

The diffusion theory describes the migration of molecules from a place with a higher concentration to one with a lower one caused by Brownian motion in accordance to the first Fickian law. The driving force in accordance to the Fickian laws is a gradient (Aguilera et al. 2004). To describe the mobility of the molecules, the diffusion coefficient D is used, which is, according to the first Fickian law, the proportionality factor between the diffusion rate and the concentration gradient (Aguilera et al. 2004). In the case of filled chocolate, the more mobile oils from the filling migrate into the solidified chocolate shell to equilibrate the oil concentration difference (Ghosh et al. 2002; K. W. Smith, Cain, et al. 2007; Khan and Rousseau 2006; Marty, Baker, et al. 2005). Migration of fat from the chocolate into the filling is also possible, but to a lesser extent (Birkett 2009; Talbot 1990; Timms 2003). Both results in a hardening of the filling and a softening of the chocolate (Ziegleder 2005a). The thermodynamic transport mechanism of diffusion in chocolate is the difference in fat content and composition. Every TAG is migrating due to its specific concentration difference between the two phases chocolate and filling. So, at least, the TAG fingerprint of chocolate fat and filling oils are constantly changing and converge. This was demonstrated by high performance liquid chromatography (HPLC)-measurements during storage (Ziegleder 2002).
To describe migration over time, Ziegleder proposed a simplification of the solution of the second Fickian law, which is given in eq. 2.4.1 (Aguilera et al. 2004; Galdámez et al. 2009; Ghosh et al. 2002; Miquel et al. 2001; Ziegleder, Petz, et al. 2001).

$$\frac{m_t}{m_s} = \frac{A}{V} \cdot \sqrt{D_{eff} \cdot t} \tag{2.4.1}$$

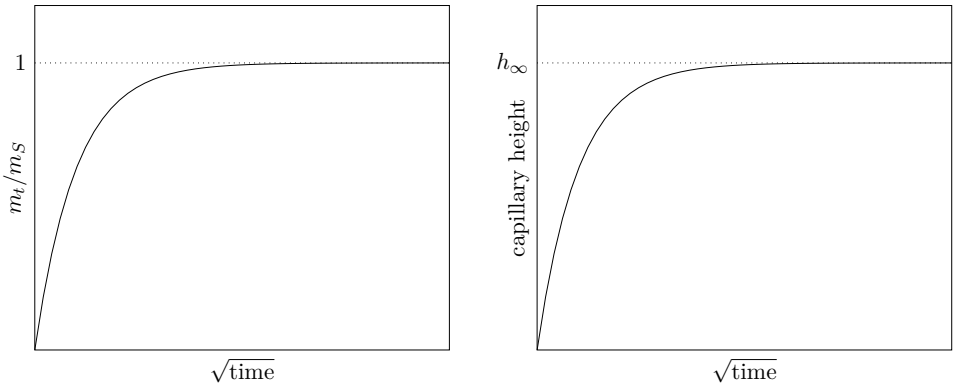
With m_t being the amount of migrated oil at a certain time t and m_s the migrated amount at saturation. The contact area between the chocolate and the filling is A, the effective diffusion coefficient is D_{eff} and V is the chocolate volume. The impact of shell thickness is described by the specific length $l = \frac{A}{V}$ (Ziegleder, Moser, et al. 1996a).
Chocolate is a heterogeneous systems, consisting of fat, which is partially solid at room temperature and enclosed sugar and cocoa particles. Therefore the effective diffusion coefficient D_{eff}, given in eq. 2.4.2, is used in this work. It is affected by the molecular diffusivity D_0, the volume fraction of the liquid fat in the chocolate Φ, given in eq. 2.4.3, and the tortuosity τ as given in eq. 2.4.4. Tortuosity is used to describe diffusion and fluid flow in porous media, e.g. particle filled media and is defined as the square of the ratio of the actual flow path length l_{eff} to the straight distance l between the ends of the flow path.

$$D_{eff} = D_0 \cdot \frac{\Phi}{\tau} \tag{2.4.2}$$

$$\Phi = \frac{V_{fat}^{liq}}{V_{choc}} \tag{2.4.3}$$

$$\tau = \left(\frac{l_{eff}}{l}\right)^2 \tag{2.4.4}$$

The diffusion can be decelerated by increasing the tortuosity. This can be done by adding particles, which extends the diffusion path way (Galdámez et al. 2009). However, the composition of chocolate is limited by regulatory framework and production parameters, which allow only small changes regarding tortuosity. Thus, the volume fraction of the liquid phase Φ is the main influencing factor for diffusion through the fat phase. The particle size of the cocoa and sugar particles and the degree of tempering have a minor effect with regards on migration (Ziegler 2009).
In the beginning the migrated amount is directly proportional to the square root of time. During further storage migration decelerates and approaches saturation as can be seen in fig. 2.11a. Eq. 2.4.1 is suitable to describe the migration in the beginning. However, using the simplified solution of the Fickian law and a constant diffusivity, it is not possible to describe the spatial distribution of oil in chocolate (Choi, K. L. McCarthy, and M. J. McCarthy 2005). Additionally it is not suitable to describe the saturation due to the root function, which has no horizontal asymptote. The difficulties in describing the whole migration process from the beginning until the end are mainly caused by the complex microstructure of multi-component food systems, which might change during processing and storage (Aguilera et al. 2004; Ghosh et al. 2002; Greiner et al. 2014). Therefore variable effective diffusivity constants D_{eff} have to be determined empirically in stead of using constant ones (Aguilera et al. 2004). This discrepancy supports the hypothesis that fat migration is based on additional or other mechanisms, such as capillary flow (Aguilera et al. 2004).



(a) Diffusion through the fat phase (b) Capillary diffusion

Figure 2.11: Migration over time based on diffusion through the fat phase, which ends in a partition equilibrium and capillary diffusion, which ends in an equilibrium of forces

2.4.2 Fat migration caused by capillary flow

Capillary flow is another mechanism used to describe filling oil migration in chocolate (Aguilera et al. 2004). The capillary effect describes the rise of a fluid in a capillary column caused by surface tension (Ziegler 2009).
Chocolate consists of solids dispersed in a continuous fat phase (Quevedo et al. 2005). During crystallization of the cocoa butter in chocolate cracks and crevices might form. The fraction of solids is increased by solidified cocoa butter. The remaining liquid cocoa butter is soaked up by capillary forces in the interspace between the solids, including cocoa and sugar particles as well as fat crystals. This leads to the assumption that, with regards to oil migration, all pores in chocolates are filled with liquid oil due to the capillary effect (Aguilera et al. 2004).
The Lucas-Washburn equation, given in eq. 2.4.5 describes the rise of a fluid from an infinite reservoir in a cylindrical capillary column.

$$\frac{2}{r} \cdot \gamma \cdot \cos\Theta = \frac{8}{r^2} \cdot \mu \cdot h \cdot \frac{dh}{dt} + \rho \cdot g \cdot h \tag{2.4.5}$$

The capillary flow is besides others affected by gravitation and friction. The parameters are radius r, surface energy γ, contact angle between fluid and surface of the capillary Θ, viscosity of the fluid μ, density of the fluid ρ, and gravitational acceleration g resulting in height of capillary rise h in dependency of time t (Aguilera et al. 2004; Ziegler 2009). High surface tension, low density of the fluid and a small radius of the capillary column result in a high capillary rise.
The time dependent height of the migrating oil caused by capillary flow is shown in fig. 2.11b. In the beginning the rise of a liquid in a vertical capillary is linearly proportional to the square root of time (Zhmud et al. 2000). After further storage the height

of capillary rise reaches a maximum at which the hydrostatic pressure is in equilibrium with the pressure caused by surface tension (Aguilera et al. 2004). Thus, the two mechanisms can not be distinguished according to their time response.
The definition of chocolate as a dispersion of cocoa and sugar particles as well as solid fat crystals dispersed in a liquid oil phase is well-accepted but precise information about the microstructure of chocolate is unavailable (Loisel et al. 1997). However, the difference in surface tension between liquid and crystallized TAG is small and the shape of the capillary columns in chocolate might not be round. Rough or heterogeneous particle surfaces, as for instance in chocolate, might cause complex contact angle phenomena (Walstra 2009).

2.5 Manufacture of chocolate products

2.5.1 Cocoa and chocolate processing

The cacao tree (Theobroma cacao, L.) originates from South and Central America. It was introduced in Asia in the sixteenth and seventeenth century and in West Africa in the eighteenth and nineteenth century. These three, West Africa, South East Asia and South America, are still the main cocoa growing areas. The different growing conditions lead to varying fat compositions of the cocoa butter. Additionally there are many varieties of cocoa. The most famous ones are Criollo, Forastero and Trinitario, which started as a hybrid of Forastero and Criollo. They vary in cocoa bean color, size and flavor as well as resistance to pests and diseases. All varieties have in common, that their fruits, the cocoa pods, grow from the trunk and thicker branches. (M. S. Fowler 2009)
Cocoa processing starts with cocoa pod harvesting. It is important that the pods are ripe, otherwise mono- and diglycerides are present due to incomplete TAG synthesis. On the other hand in overripe pods enzyme reactions lead to TAG degradation to mono- and diacylglycerols. The pods are broken and the husk is removed, while beans and pulp are fermented together (Afoakwa 2010). During fermentation sugars and mucilage are disintegrated by natural yeasts and bacteria, which multiply in the pulp. The pulp reduces into a fluid state and drains off. Afterwards, the fermented beans are dried to a moisture content of 50 mg/g to 80 mg/g to avoid mildewing during transportation (Minifie 1989). The dried beans are transported to Europe or the US for further processing. The next steps, roasting and grinding, can be performed in two ways. In most cases the nibs are roasted, but whole bean roasting is also possible (Beckett 2000; Minifie 1989). Whole bean roasting can lead to inhomogeneous roasting results due to unequal size of the beans, but facilitate removing of the skin or testa (the shell). Nib roasting requires a breakdown of the still elastic beans, but leads to more homogeneous roasting. The roasted nibs or beans are finely ground to obtain cocoa liquor. A scheme of the manufacturing process is shown in fig. 2.12.
Chocolate consists of cocoa liquor, cocoa butter, sugar and possibly milk powder. In most countries the amount of these main components and the usage of other ingredients is regulated. The three main kinds of chocolate are dark chocolate, milk chocolate and white chocolate. The ingredients are mixed, while some of the cocoa butter is restrained. The premix is reduced to small pieces using a roll refiner. The particle size of chocolate should be less than 30 µm to avoid a sandy mouthfeel. On the other hand, particles should not be too small to reduce the amount of cocoa butter needed. The particle size and the amount of cocoa butter influence flow properties. Flow properties are not only important for the taste but also for manufacturing. After roll refining the now powdery mixture is treated in the conche. Thereby agglomerates are dissolved, aroma diffusion takes place and the remaining ingredients, cocoa butter and lecithin are added. After conching the chocolate contains all ingredients and flavor is fully developed. The ready to use chocolate needs to be tempered to achieve the stable crystal form β_V.

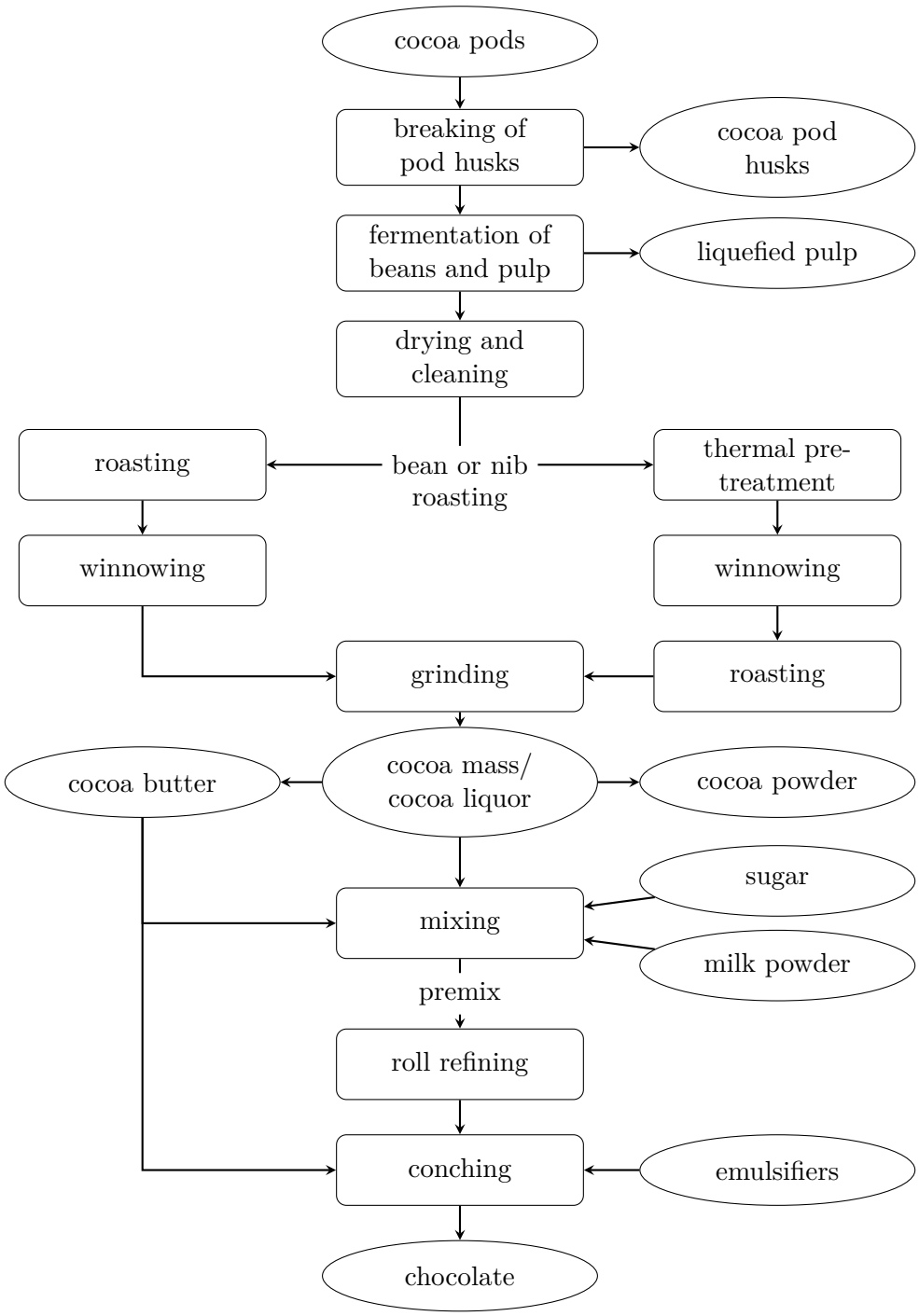


Figure 2.12: Cocoa Processing from pod to chocolate

2.5.2 Chocolate tempering

Chocolate is pre-crystallized, the so called tempering, by applying a specific temperature profile. For this purpose, the chocolate is fully melted at 50 °C followed by cooling to 27 °C. During cooling crystal nuclei are formed. Re-heating between 30 °C to 32 °C ensures that instable crystal nuclei melt and only stable crystals remain in the chocolate mass. The cooling and re-heating temperatures might need to be adjusted, depending on the type of chocolate and type of cocoa butter. Additional shearing during cooling leads to a higher amount of stable crystals.
Ideally tempered mass contains around 10 mg/g crystallized fat (Schuster-Salas and Ziegleder 1992). This can also be achieved by adding seed crystals in the appropriate crystal form to the cooled but still liquid chocolate mass (Zeng 2000). The tempering degree can be controlled by taking a sample of tempered chocolate and recording a tempering curve by measuring time and temperature upon cooling of the mass. The typical progression of a tempering curve of dark chocolate is shown in fig. 2.13 for normal, under and over tempered chocolate. From this curve, the so-called temper index (TI) can be calculated.

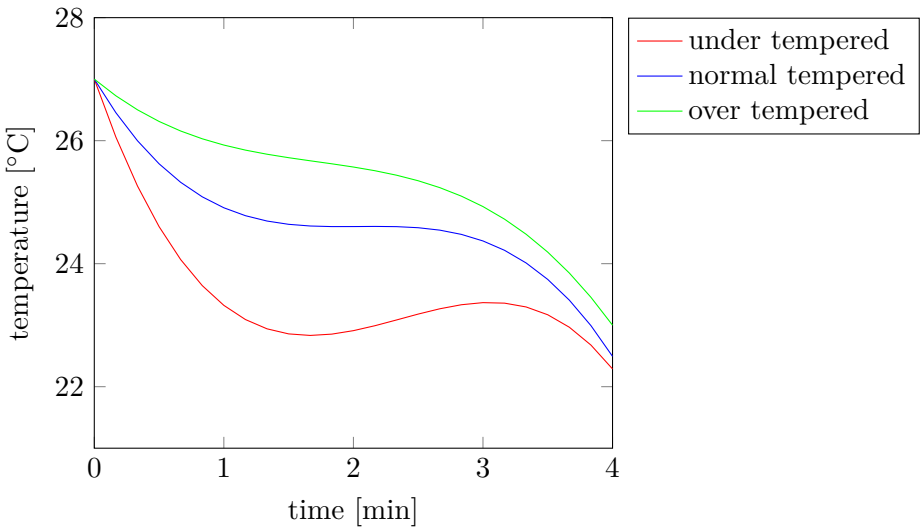


Figure 2.13: Exemplary cooling curve of under, normal and over tempered dark chocolate for TI determination

Due to the increasing amount of solids tempering affects the flow properties (Danzl and Ziegleder 2013). Flow properties are important for certain forming techniques, which are described in the following chapter. After forming the chocolate is cooled to solidify and afterwards demolded and packed.

2.5.3 Forming filled chocolates

Conventional shelling There are different ways to produce filled chocolates. The oldest, easiest and therefore most used technique is forming shells and filling them. In the conventional shelling or spinning process chocolate is poured into molds, shaken to remove air bubbles and turned around to remove surplus chocolate from the inside. A thin shell remains in the molds, which is than cooled, filled with the filling and sealed with tempered chocolate. The principle of conventional shelling can be sen in fig. 2.14. This is an easy way to produce shells, which offers a high variability for product design. However, the shell thickness is uneven and the removed chocolate is used as rework. This means that the chocolate has to be molten and tempered again before it can be re-used.

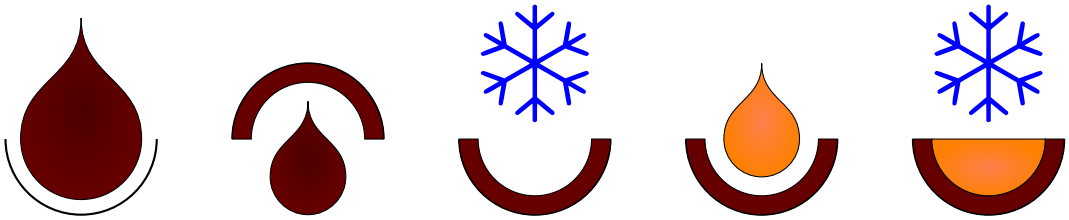


Figure 2.14: Schematic drawing of conventional shelling principle

Cold stamp Another way to produce shells is cold forming, where a cooled plunge is used. This process was invented by Boyd and Yates 1923. The usage of this technique was established around 1990 when Aasted developed the "frozen cone", which is based on the same technique (Aasted 1994). A defined amount of tempered chocolate is poured into a mold. A cooled plunge is entered into the chocolate and pushes the chocolate aside. The result is a shell with equal thickness. The shell can be filled and put together with another shell or closed by covering the filling with tempered chocolate, to obtain a filled chocolate product. A low temperature of the plunge is important to ensure that the chocolate does not stick to the plunge, but lowering the temperature is also limited. At too low temperatures the chocolate solidifies amorphous, which results in low contraction and impeded demolding. For industrial manufacturing plunge temperatures vary between $-20\,°C$ to $5\,°C$ for $3\,s$ to $180\,s$ (Böhme et al. 2003). Due to the low temperatures a dry air environment is needed. Another disadvantage is the supplementary cooling effort for the plunge. In addition high dimensional accuracy is needed, whereby variability of product design is reduced due to the high costs for the plunges. On the other hand there is no rework during the process and it is almost independent from chocolate flow properties. The schematic of the processing principle can be seen in fig. 2.15.

Enrobing Beside these two methods, where a shell is produced and filled, the manufacturing of a so called core or center piece, which is enrobed with chocolate afterwards, is possible. The center piece is brought to a certain temperature and placed on a wire belt.

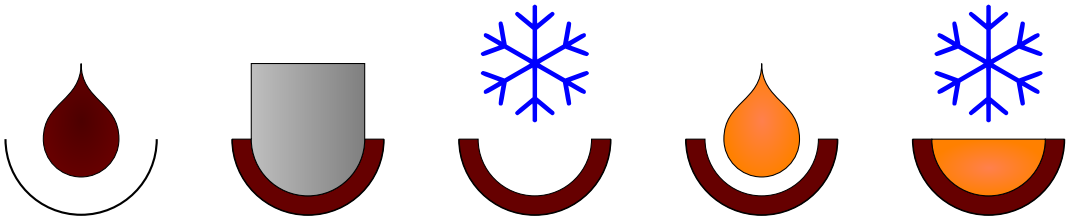


Figure 2.15: Schematic drawing of cold forming principle

It passes a chocolate curtain where the top and the sides are covered with chocolate. The surplus chocolate can be collected in a drip pan, through which the center piece is moved to cover its bottom (not shown) or the bottom is covered by using a roller, as can be seen in fig. 2.16. An air blower might be used to remove any remaining chocolate, which then flows back into a sump from which it is reused. To achieve an equal shell thickness, a glossy surface and to avoid the formation of a chocolate tail at the bottom of the product, the flow properties need to be controlled. The temperature of the center piece is also important. If the center piece is too hot, the crystals in the tempered chocolate melt; if it is too cold the chocolate will solidify too fast resulting in a thick shell.

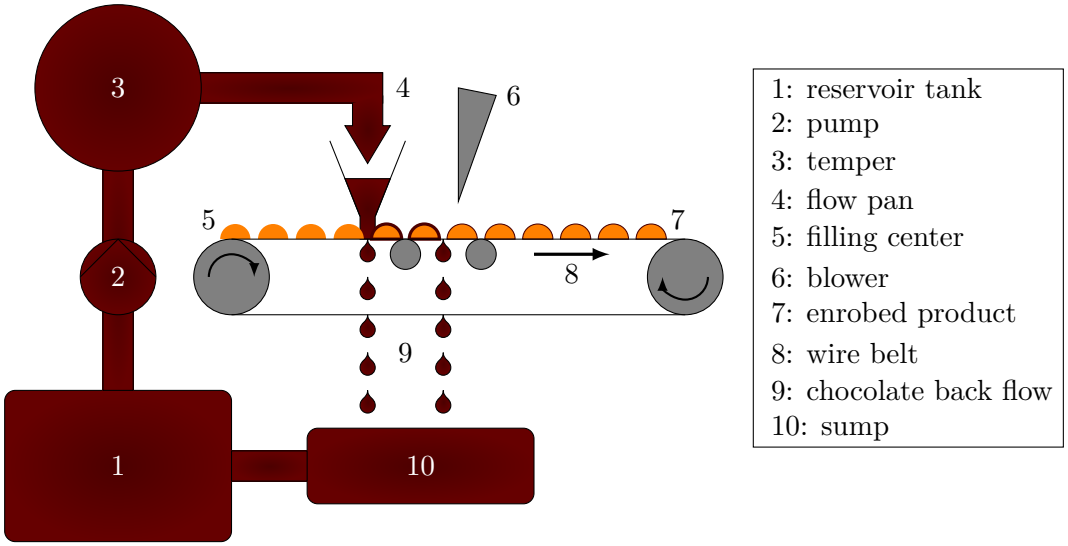


Figure 2.16: Schematic drawing of enrobing principle

One-Shot The methods described until now always include two steps: forming a shell or a center and adding the other part in liquid state. In contrast, the one-shot or single-shot process is a way to combine a molten filling with a molten chocolate in one step. This technique was already used around 1930, but was just spread around 1970, when

the use of computers enabled sufficient controlling (Minifie 1989). For the production, a concentric nozzle is used to co-inject the shell and the filling at the same time. The chocolate is filled through the outer part and the filling through the inner part of the nozzle, as can be seen in fig. 2.17. The precise control of the filling process as well as the matching of the ingredient flow properties are important (Padar 2009). The variability of the fillings is limited due to the flow properties and the direct contact between filling and chocolate. A filling which melts at a high temperature is unsuitable because the crystals in the tempered chocolate would melt. Thus, using a one-shot process requires knowledge about the process itself and about the flow properties of the ingredients. The product variability is limited by the flow properties and the unintentional mixing of chocolate and filling during production results in a softer shell (Ziegleder 2005b). However, only one cooling tunnel is needed, which makes the process energy and time saving.

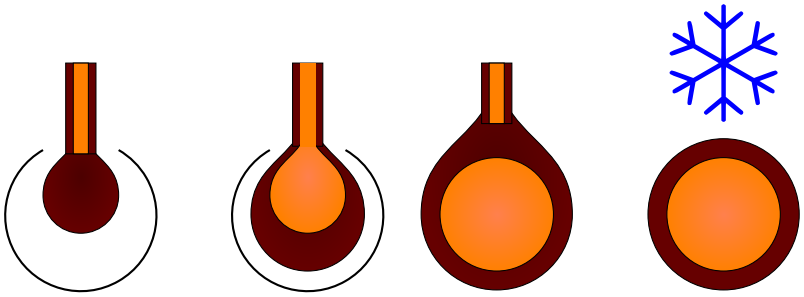


Figure 2.17: Schematic drawing of one-shot principle

2.6 Fat bloom

The main consequence of the interaction of crystallization and migration is the formation of fat bloom. Fat crystals at the surface of chocolate products cause light scattering. At the beginning, this reflection causes a dull surface, resulting in a whitish layer over time. This layer appears in different degrees and shapes and is called fat bloom (Becker 1957; Ziegleder, Geier-Greguska, et al. 1994; Afoakwa, Paterson, M. Fowler, and Vieira 2009a). Sugar bloom also forms a whitish layer on the surface, but the causes are different. Sugar bloom is mainly caused by high humidity, when sugar is dissolved and recrystallizes on the surface.

2.6.1 Causes for fat bloom

According to literature, there are four main causes, which are described below, for fat bloom formation, which are all a result of migration, crystallization or their interaction with regard to microstructure (Ziegleder and Mikle 1995a; Khan and Rousseau 2006).

2.6.1.1 Post-crystallization

As already mentioned in chap. 2.2, crystallization can be divided in three steps: nucleation, crystal growth and post-crystallization. In the first step, around $2\,cm^3/m^3$ to $20\,cm^3/m^3$ of the fat crystallize. Depending on the used cocoa butter, the amount of crystallized fat increases to $0.45\,m^3/m^3$ to $0.60\,m^3/m^3$ during crystal growth and reaches values of $0.65\,m^3/m^3$ to $0.80\,m^3/m^3$ during further storage (Cebula and Ziegleder 1993; Försterling et al. 1981). The last post-crystallization step is slow and therefore large crystals can be formed. These crystals might become visible at the surface as fat bloom (Ziegleder and Mikle 1995b). The formation of large crystals is also promoted by Ostwald ripening, which causes the further growth of large crystals at the expense of smaller ones (Afoakwa, Paterson, M. Fowler, and Vieira 2009a). The physical background of Ostwald ripening is the stabilization due to a reduced surface energy on the larger surface with smaller curvature.

2.6.1.2 Polymorphic transition

During further storage, crystallization continues and a polymorphic transition from β_V to β_{VI} crystal form occurs (Cebula and Ziegleder 1993). The primary idea for the origin of fat bloom formation was that the needle shaped β_{VI} crystals pierce through the surface and become visible (Vaeck 1960). However, Bricknell and Hartel 1998 showed that sole polymorphic transition is not crucial for fat bloom development. Nonetheless, it was observed that fat bloom development is often accompanied by polymorphic transition (K. W. Smith, Cain, et al. 2007). Additionally, polymorphic transition was only observed in dark chocolate, while milk chocolate remains in the β_V crystal structure but fat bloom can also appear on milk chocolate (Cebula and Ziegleder 1993; Ziegleder 1993a). It should be noted that polymorphic transition as well as fat bloom formation are aging processes of chocolate. However, the formation of fat bloom and the occurrence of polymorphic transition are not always coupled.

2.6.1.3 Fat fractionation

Fat fractionation or demixing is a consequence of the before mentioned processes: post-crystallization, Ostwald ripening and polymorphic transition. The fast crystallization process of tempered chocolate leads to the incorporation of TAG, which are usually in liquid state at the crystallization temperature, into the crystal lattice. During storage of the freshly produced pralines the TAG distribution equilibrates. Asymmetric TAG from the crystal are dissolved in the liquid phase, while symmetric TAG, especially those of the sat-O-sat type, are adsorbed at the crystal surface. During this process of fat fractionation, shown in fig. 2.18, the crystals grow and might appear as fat bloom (Ziegleder and Mikle 1995a).

Fat fractionation is particularly critical when filling fats or oils get into the chocolate unintentionally, as might be the case during the enrobing process (Rothkopf, Danzl, and Ziegleder 2016). Some TAG, e.g. OOO can be incorporated into the crystal lattice in

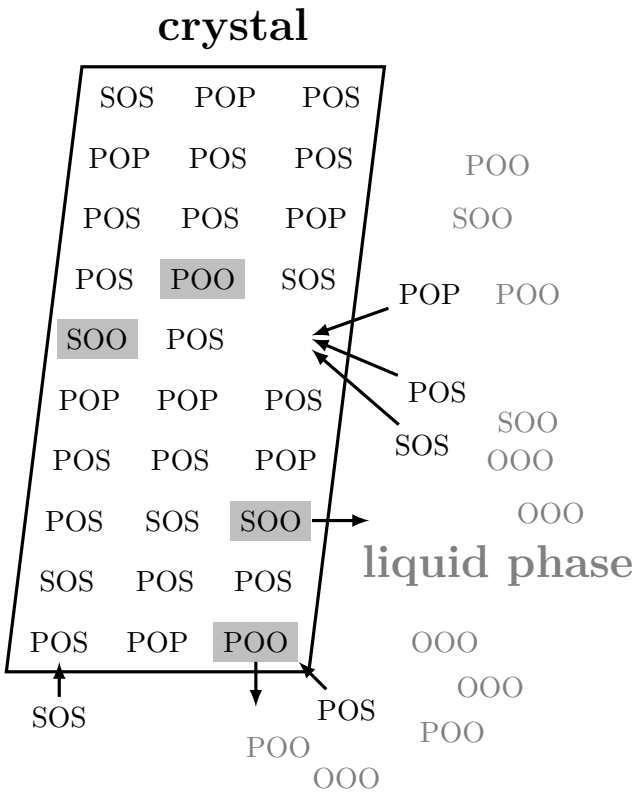


Figure 2.18: Self-fractionation of TAG according to Ziegleder and Mikle 1995a

amounts of 50 mg/g to 100 mg/g (Rothkopf and Danzl 2015; Ziegleder, Geier-Greguska, et al. 1994). They are then gradually removed from the crystal network (Rothkopf, Kind, et al. 2017).

2.6.1.4 Migration fat bloom

It can be observed that filled products are particularly susceptible to fat bloom formation. This is mainly caused by migration of oils from the filling into the surrounding chocolate. Therefore this type of fat bloom is called migration fat bloom. As a consequence migration of oils from the filling in chocolate is a highly discussed topic in scientific literature as was described in chap. 2.4.
With regards to fat bloom formation two kinds of migration have to be distinguished. In the first case, macro migration from a filling into chocolate is regarded, which has been described in chap. 2.4. Independent of the type of migration, the migrated amount is initially proportional to the square root of time. However, the explanation for fat bloom caused by migration in filled products varies. On the one hand, the migrating oil might dissolve some of the crystallized TAG, which are then able to crystallize at the surface. On the other hand, the mobility of the migrating oil has an impact. While the fat in chocolate is mainly solid, fillings often contain high amounts of liquid oil. As a result the overall migration rate from the filling into the chocolate is higher than reversely. Ziegleder and Mikle 1995a assumed, that the resulting overpressure in the shell pushes the TAG in the chocolate to the surface, where they crystallize and become visible as fat bloom.
The second kind of migration, which has to be taken into account is the micro migration of TAG. The interface of chocolate and filling is particularly susceptible (Ziegler and Szlachetka 2005; K. W. Smith, Cain, et al. 2007). Additionally, the ability of TAG to resolve in the melt and crystallize elsewhere is the transport mechanism for the before mentioned phenomenons (Beckett 2009b; Ziegleder and Mikle 1995a; K. W. Smith and van Malssen 2003).
Migration of oil from filling into chocolate is influenced by many factors: the type of filling plus the particle size and distribution have an impact (Ziegleder and Mikle 1995a; K. W. Smith, Cain, et al. 2007; Afoakwa, Paterson, M. Fowler, and Vieira 2009a). Other factors are the contact area and contact intensity between chocolate and filling as well as the volume-ratio of chocolate and filling. As already mentioned, the oil content and mobility have to be considered, too (Birkett 2009). The latter are affected by temperature, which has a major impact on fat bloom formation.
Regarding migration fat bloom, a second overlaying effect can be observed. The migrating oil might additionally accelerate the β_{V} to β_{VI} transition, which can go along with fat bloom formation (K. W. Smith, Cain, et al. 2007).

2.6.2 Impact factors for fat bloom formation

The four factors known from the literature, namely post-crystallization, polymorphic transition, fat fractionation and migration, which were described in chap. 2.6.1, which

influence the formation of fat bloom can in turn be traced back to migration, crystallization, their interaction and their impact on microstructure. Otherwise, crystallization, migration and microstructure are affected by processing and environmental conditions. For instance, chocolates with larger particles show faster fat bloom formation than chocolates with smaller particles (Afoakwa 2010; Altimiras et al. 2007).

2.6.2.1 Fat bloom is affected by the filling

Fats that show a high SFC after solidification are usually used for enrobing masses and compounds, while fats with a lower SFC are used for fillings (Richter 2009; Vereecken et al. 2007). The fats for chocolate fillings can be divided in the following three main groups (Talbot 2009a).
Polymorphic, non-lauric fats include non-lauric oils. All fats and oils are non-lauric except palm kernel oil and coconut oil. Fats in this group show polymorphic behavior. Cocoa butter, as well as cocoa butter equivalent (CBE) belong to this group.
Non-polymorphic, non-lauric fats are often hardened or fractionated oils, such as palm oil, soy oil, canola oil or cotton seed oil. These fats are named cocoa butter replacer (CBR).
Lauric fats are based on palm kernel or coconut oil. They are often fractionated and are called cocoa butter substitute (CBS).
The filling oil should have a TAG composition which is similar to that of the surrounding mass. However, choosing a filling is usually based on its sensory properties, such as flavor, taste, texture and on quality and shelf-life aspects (Talbot 2009a).
The use of incompatible fats is especially critical, since eutectic effects can occur, which soften the chocolate to an immoderate extent (Aguilera et al. 2004; Bigalli 1988; Talbot 1990). An exception is milk fat (e.g. in milk chocolate), which is known to be incompatible with cocoa butter, but also reduces fat bloom formation in small amounts (Kleinert 1997; Ziegleder 1993a; Ziegleder 1993b; Sonwai and Rousseau 2010). The migration rate also depends on the molecule size. Small TAG migrate faster than long-chained ones (Delbaere et al. 2016).

2.6.2.2 Processing parameters

The crystallinity and oil mobility in the filling might also affect migration. However, the tempering regime seems to have no effect on fat bloom formation (Juul 2010). A major influence on bloom tendency is caused by the processing speed. When the chocolate shell is not fully crystallized before filling early migration may be forced. This is also found in one-shot pralines where the liquid masses are in contact during processing and mixing at the interface might occur (Ziegleder, Danzl, et al. 2011). NMR measurements showed that it takes several minutes for a dark chocolate (containing 0.337 g/g cocoa butter from West Africa and 0.02 g/g anhydrous milk fat) to reach a crystallinity which is high enough to resist against migration of filling oils (Strassbourg et al. 2006). In case of cold forming, post-tempering of the shells before filling might increase shells stability (Juul 2010). Enrobed products are particularly susceptible to fat bloom formation due

to a partial contamination of the chocolate mass with filling oils during the process (Rothkopf, Danzl, and Ziegleder 2016).

2.6.2.3 Storage temperature

Depending on the storage temperature the impact of either crystallization or migration predominates, as can be seen in fig. 2.19. At higher temperatures, migration is accelerated due to higher mobility of the migration phase and less resistance of the chocolate, caused by a reduced SFC (Ali et al. 2001; Altan et al. 2011; Dahlenborg 2014; Guiheneuf et al. 1997; Khan and Rousseau 2006; Lonchampt and Hartel 2004; Miquel et al. 2001; Ziegleder and Schwingshandl 1998). At lower temperatures, the crystallization is predominant, which leads to a more intense crystallization of the fat on the surface, which becomes visible as bloom. A fluctuating temperature leads to a higher migration at higher temperatures and a higher intensity of fat bloom crystallization on the surface at lower temperatures (Timms 1984; Ziegleder and Schwingshandl 1999).

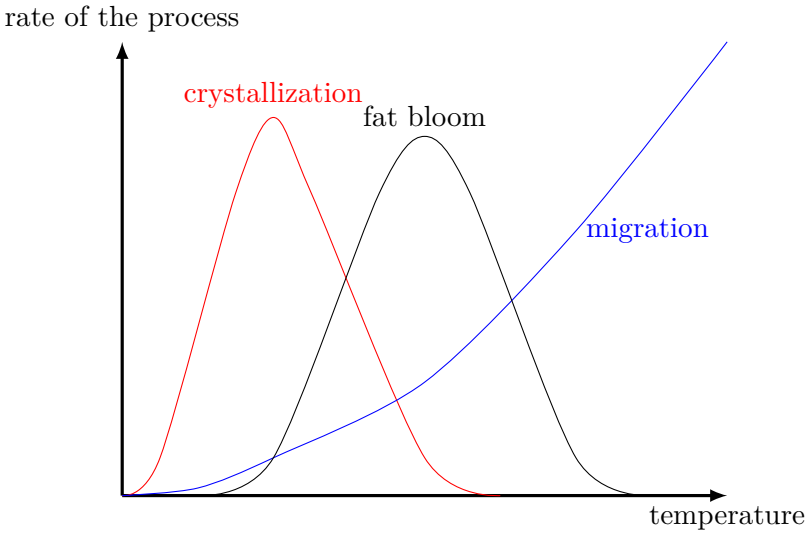


Figure 2.19: The optimum temperature for fat bloom formation in filled chocolate products is in between the optimum temperatures of crystallization of fat bloom on the surface and high migration of oils from the filling according to Ziegleder and Schwingshandl 1999

For shelf life prediction, accelerated storage tests are used. These include temperature variations, namely isothermal or cycling temperature storage, but humidity variation is also possible (Ali et al. 2001; Bricknell and Hartel 1998; Depypere et al. 2009; Guiheneuf et al. 1997; Jin and Hartel 2015; Jinap et al. 2009; Khan and Rousseau 2006; Nightingale et al. 2011; Ziegleder and Schwingshandl 1999; Rothkopf, Schütz, et al. 2017).
In the case of *isothermal storage*, the optimum temperature has to be found, where migration rate is high enough to bring TAG to the surface and low enough, so that

they are able to crystallize there. In contrast, *cycled storage* temperatures promote migration during high temperature periods and accelerate crystallization during low temperature periods (Cartier 2009; Subramaniam 2016). Both tests are used in the industry. However, to distinguish the effects of migration and crystallization on fat bloom formation, a mathematical description must be possible. This is only the case for isothermal storage.

2.6.3 Production parameters and fat bloom formation

Due to the interaction of crystallization and migration, the production and storage parameters have a major impact on fat bloom stability (Ziegleder and Mikle 1995b; Ziegleder and Mikle 1995a; Ziegleder and Mikle 1995c). The different shell forming techniques vary in technical equipment, energy consumption, production time and in shelf life. Ziegleder, Danzl, et al. 2010 compared different manufacturing methods for filled pralines: conventional shelling, cold forming, one-shot and enrobing. Considerable differences were found in SFC of fresh products, filling fat migration during storage and fat bloom appearance.
The results for fat bloom stability found for pralines produced with different techniques lead to the assumption that initial filling fat in the chocolate and the amount of crystallized cocoa butter when the filling is added are the reasons for the differences. The comparison of pralines with preformed shells and the time for stabilization before filling shows that shells with a higher amount of crystallized cocoa butter are more stable with regard to fat bloom. This also brought up the idea to stabilize shells by melting instable crystals by warm post-tempering before filling (Juul 2010).
Another possibility of warm post-tempering is the storage of filled pralines at higher temperatures. This leads to a fast migration (Ziegleder 1995). It is assumed that above a certain concentration the fat is too soft to crystallize on the surface. This might also be the case with one-shot pralines, where parts of the filling and chocolate unintentionally mix during dosage. Beside accelerated migration, the surface might be sealed with fat, especially in enrobed products. In addition, mechanical tensions may cause cracks and crevices in the product. Their reduction might also reduce fat bloom formation (Minifie 1989).

3 Material, processes and methods

3.1 Sample material

A dark chocolate was offered by an industrial partner and used as a model chocolate for all experiments. Four cocoa butter samples were provided by industrial partners. Different fillings as well as filling fats and oils were used in this work. All sample materials are listed in tab. 3.1.

Table 3.1: Fillings and filling fats and oils for experimental procedures

sample	name / supplier / description
dark chocolate	39 g/100g cocoa mass, 13 g/100g cocoa butter, 47.5 g/100g sugar, 0.5 g/100g lecithin
CB 01	Astra A, DeZaan, ADM Inernational Sarl, The Netherlands, origin: West Africa
CB 02	no information available
CB 03	no information available
CB 04	origin: West Africa
hazelnut oil	Vom Fass AG, Waldburg, Germany
butterfat	Butaris, Hoche Butter GmbH, Uelzen, Germany
coconut fat	Palmin®, Peter Kölln KGaA, Elmshorn, Germany
almond oil	Vom Fass AG, Waldburg, Germany
olive oil	Bertolli - Unilever Deutschland GmbH, Hamburg, Germany
walnut oil	P. Brändle GmbH, Ölmühle - Speiseölgroßhandel, Empfingen, Germany
nougat	Nougat Extraklasse NNXX, Lubeca Lübecker Marzipan-Fabrik v. Minden & Bruhns GmbH & Co KG, Stockelsdorf, Germany

3.2 Investigation of particle impact on chocolate crystallization

The solid fat content (SFC) and crystallization behavior of all chocolate and cocoa butter samples was determined as described in chap. 3.6. To study the impact of particles on crystallization and migration, chocolate was compared to cocoa butter and cocoa butter mixtures. The mixtures contained cocoa butter CB 04 with either fat-free cocoa powder (D-00-ZR, ADM International Sarl, Rolle, Swiss) or finest sugar (SF0, Pfeifer & Langen, Cologne, Germany) or both. An additional second series with soy lecithin powder

(Cargill Texturizing Solutions Deutschland GmbH & Co. KG, Hamburg, Germany) was prepared. For this purpose one third of lecithin powder was mixed with two thirds of cocoa butter. This premix was added to the model systems to achieve 0.5 g/100g lecithin based on total sample weight. The aim was to identify the impact of particle surfaces and surface active ingredients on cocoa butter crystallization.

3.3 Investigation of filling lipids in chocolate and cocoa butter

3.3.1 Impact of filling fats and oils on cocoa butter and chocolate crystallization

Mixtures of filling fats and oils in chocolate or cocoa butter were investigated using physical and chemical methods as described in chap. 3.6. The aim was to study the impact of filling fats and oils on crystallization behavior.

3.3.2 Proof of the presence and quantification of filling lipids in chocolate

Binary mixtures of chocolate with hazelnut oil, butterfat and coconut fat were used to establish methods for detection and quantification of filling fat and oil in chocolate. Hence, decision limit and determination limit were calculated in accordance to DIN 2008. The decision limit is the smallest amount of filling fat, which leads to a significantly different signal of the analytical system compared to a sample without filling fat. In contrast, the determination limit is the lowest quantifiable content of filling fat, which is always linked to numerical data.
The fatty acid (FA) composition of some of the binary mixtures with dark chocolate was determined using the gas chromatography (GC) method described in chap. 3.6.1.
To verify the method, the recovery rate RR was calculated using eq. 3.3.1 (Kromidas and Kuss 2008).

$$RR = \frac{\overline{x}}{x_T} \tag{3.3.1}$$

With $\overline{x}$ being the measured mean value and x_T being the target amount, given by the sample preparation, e.g. by the preset concentration.

3.4 Filling oil migration in cylindrical chocolate model systems

The migration of oil from the filling into the surrounding chocolate can be described both by diffusion through the fat phase (Fickian law) and by capillary flow (Lucas-Washburn equation). In both cases the migrated amount in the beginning is proportional to the square root of time (Aguilera et al. 2004). Migration is influenced by many factors, which can be explained by both mechanisms. Therefore a lot of models have been developed to study migration in chocolate. They all have in common that two different components are brought into contact, trying to copy a filled chocolate product. An example is the

"'Washer"' test introduced by Talbot 1996 and used by several other researchers (K. W. Smith, Cain, et al. 2007; K. W. Smith 2008; Ghosh et al. 2002). It is performed by using thin chocolate plates and placing them directly on the filling or on a filter paper soaked up with oil (Ziegler, Shetty, et al. 2004; Tran et al. 2015). This setup was adapted for this study and the composition of the cylinders was adjusted to clarify the open questions regarding migration.

3.4.1 Cylindrical model systems

A setup of a cylindrical model system, which can be seen in fig. 3.1 according to K. W. Smith, Cain, et al. 2007 was created to study filling oil migration in chocolate and cocoa butter. Nougat was chosen as the filling for all trials as the detection methods were most effective for this type of filling (see chap. 4.2).

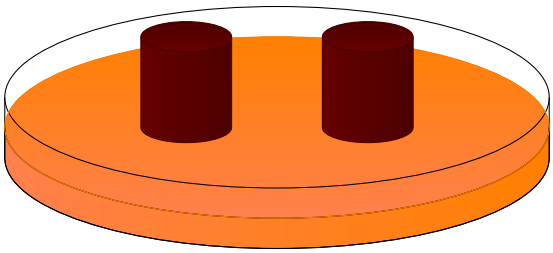


Figure 3.1: Setup of cylindrical model systems

While the nougat base, which contains hazelnut oil, remained constant throughout all experiments, the cylinders consisted of different mixtures of dark chocolate, cocoa butter (CB 04), fat-free cocoa powder (D-00-ZR, ADM International Sarl, Rolle, Swiss) or finest sugar (SF0, Pfeifer & Langen, Cologne, Germany). The cylinder variation is shown in fig. 3.2 and 3.3.

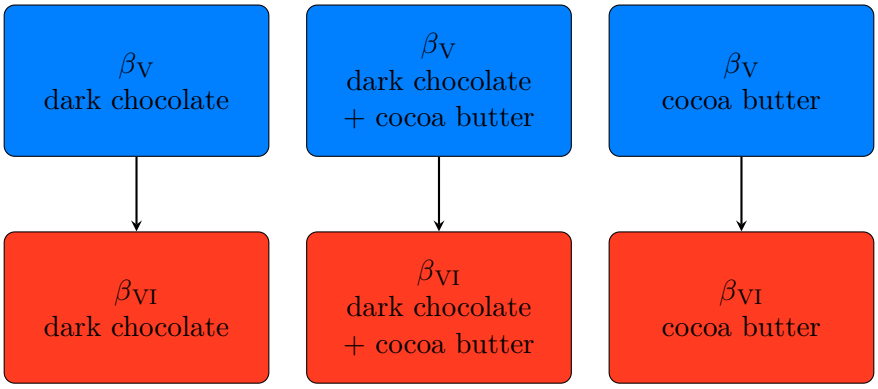


Figure 3.2: Basic experiment (blue) and changed crystal form cylinders (red) for model migration measurements, see text for detailed explanation

The **basic experiment**, whereof all other variations were derived, is shown in fig. 3.2 in blue. It included cylinders of dark chocolate, cocoa butter CB 04 (see section 3.1) and a 1:1 mixture of dark chocolate and cocoa butter CB 04 , all in β_V crystal modification. The main difference in this basic setup is the portion of cocoa and sugar particles. This setup is used to identify the main migration type, which is either along the particle surface or through the fat phase.
Further studies on the continuous phase impact were carried out with **changed crystal form cylinders** where crystals in a higher polymorphic form were present. Another batch of cylinders was prepared as described for the basic experiment with dark chocolate, cocoa butter and a 1:1 mixture. To evaluate the impact of the crystal structure, these cylinders were stored at 27 °C to accelerate crystal transition. The presence of β_{VI} crystals was verified using differential scanning calorimetry (DSC) melting curves as described in chap. 3.6.3.1.

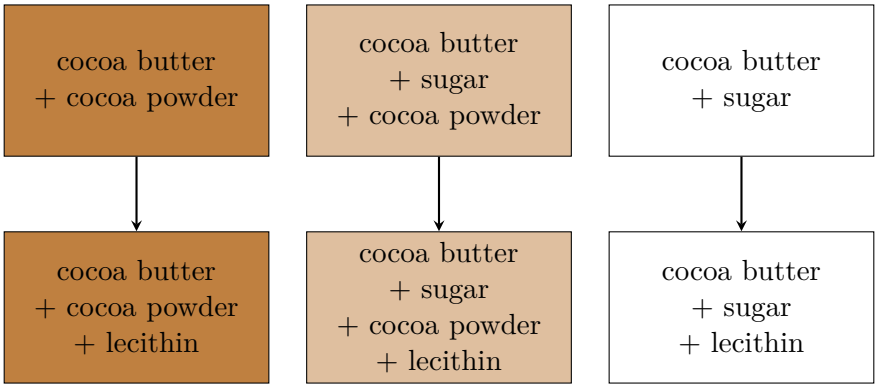


Figure 3.3: Cylinder variation for model migration measurements with different types of particles and lecithin addition, see text for detailed explanation

Since the cocoa butter in dark chocolate and the pure cocoa butter in the basic experiments do not necessarily have the same chemical composition, a model chocolate system with changed **dispersed phase** was prepared, shown in fig. 3.3. For this purpose, cocoa butter CB 04 was mixed with either cocoa powder or finest sugar or both and in a further approach lecithin was also added to the system, to alter the particle surface. The names of the samples are shown in tab. 3.2.

Table 3.2: Naming and composition of the samples of cocoa butter mixed with different amounts of finest sugar (S) and fat-free cocoa powder (CP)

model system	sugar [g/g]	cocoa powder [g/g]
S30_CP0	0.30	0.0
S50_CP0	0.50	0.0
S10_CP20	0.10	0.20
S25_CP25	0.25	0.25
S0_CP30	0	0.30
S0_CP50	0	0.50

To produce the different types of cylinders, the components were heated to 40 °C and optionally combined and homogenized with a magnetic stirrer. For cylinder preparation, the mixtures were cooled at room temperature to 29.0 °C to 29.3 °C and seeded using cocoa butter seed powder SEED 100 (Uelzena eG, Uelzen, Germany) with β_V crystals. The amount of seed powder was 5 mg/g based on the fat content.
The seeded mixtures were each poured in Nunc® 24 well plates, which were tempered at 27 °C. The plates were vibrated on a vibrating table (Pferrer Vibrationstechnik, Georgenberg, Germany) to remove air bubbles. Subsequently samples were cooled at 4 °C for 20 min and stabilized for at least 24 h at 18 °C before demolding. The resulting cylinders had a height and diameter of 15 mm each.
Per each experimental setup two cylinders were prepared and placed on a nougat base. For the nougat base, which was kept constant throughout all experiments, nougat was melted at 27 °C. A spoon was used to fill the nougat into round petri dishes with 90 mm diameter. The filled dishes were vibrated to apportion the nougat and to remove air bubbles. The prepared nougat base was stored for at least 24 h at 18 °C. To complete the sample preparation each two cylinders were placed on the nougat base in one petri dish. Therefore the plain front side of the cylinders, which was inside the mold, was put on the nougat and gently pressed against it for 5 s to ensure good contact between the surfaces. The samples were stored at 18 °C, 20 °C and 23 °C for 2, 4, 8 and 12 weeks. At the end of the storage period, the cylinders were removed from the nougat base and cut in slices of 5 mm perpendicular to the longitudinal axis using a sharp carpet knife, as shown in fig. 3.4. The samples for DSC and GC measurements were taken from the middle of each slice. The rest of the slices was used for nuclear magnetic resonance (NMR) measurements.

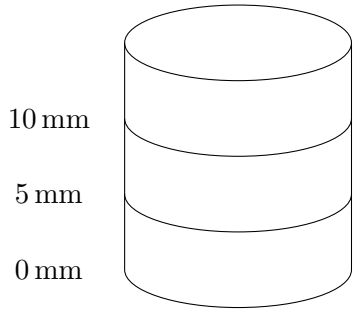


Figure 3.4: Slicing of the cylindrical model systems for sampling

3.5 Manufacture of filled model pralines

Filled model pralines were produced using a praline plant and the cold forming process. The aim of these trials was to be able to transfer the findings from the previous tests to industrial reality.

3.5.1 Tempering of chocolate

First of all, the chocolate had to be tempered, as described in chap. 2.5.2. For this purpose a Minitemper Turbo TFD 100 (Sollich KG, Bad Salzuflen, Germany) was used. To ensure correct tempering, the temper index (TI) was measured using a MultiTherm™ TC (Bühler AG, Uzwil, Swiss) and a Tempermeter E4 (Sollich KG, Bad Salzuflen, Germany). The target TI, an empirical value from the industries, was 5.0, which indicates good tempering for dark chocolate (Bühler n.d.).

3.5.2 Cold forming of model praline shells

A praline plant ubi® PN-Y (ubitec GmbH, Bergneustadt, Germany) was used for producing cold formed shells. The principle of the cold forming process is explained in chap. 2.5.3. The output of filled products is 5 kg/h to 10 kg/h and the dimensions of the plant are ca. 4 m in length and 0.7 m width (Ubitec GmbH 2012).
The stations of the praline plant used for manufacturing are schematically shown in fig. 3.5. The production starts with the feed-in of molds, where 10 molds are stocked above each other. The used molds were made from polycarbonate by Hans Brunner GmbH (Glonn, Germany). The mold, which is in the lowest position in the stock is advanced for transport to the mold preheater. The preheated molds are filled with chocolate by sink head 1, which moves towards the single cavities of each mold. There are several lanes for moving towards the cavities, which are shown in fig. 3.6. During implementation it could be shown that the two possible routes used for chocolate production did not show any differences with regard to SFC after cooling nor to fat bloom development.

At the same position as sink head 1 the pneumatic vibrator 1 shakes the molds to apportion the chocolate and to remove air bubbles. The frequency and amplitude are

<table>
<tr><td>1</td><td>feed-in of molds</td><td>49</td><td rowspan="24">cooling tunnel 3</td></tr>
<tr><td>2</td><td rowspan="2"></td><td>48</td></tr>
<tr><td>3</td><td>mold preheater</td><td>47</td></tr>
<tr><td>4</td><td rowspan="2"></td><td>46</td></tr>
<tr><td>5</td><td>45</td></tr>
<tr><td>6</td><td>sink head 1 / vibrator 1</td><td>44</td></tr>
<tr><td>7</td><td></td><td>43</td></tr>
<tr><td>8</td><td>cooled plunge</td><td>42</td></tr>
<tr><td>9</td><td rowspan="4"></td><td>41</td></tr>
<tr><td>10</td><td>40</td></tr>
<tr><td>11</td><td>39</td></tr>
<tr><td>12</td><td>38</td></tr>
<tr><td>13</td><td rowspan="4">cooling tunnel 1</td><td>37</td></tr>
<tr><td>14</td><td>36</td></tr>
<tr><td>15</td><td>35</td></tr>
<tr><td>16</td><td>34</td></tr>
<tr><td>17</td><td rowspan="2"></td><td>33</td></tr>
<tr><td>18</td><td>32</td></tr>
<tr><td>19</td><td>sink head 2</td><td>31</td></tr>
<tr><td>20</td><td>vibrator 2</td><td>30</td></tr>
<tr><td>21</td><td rowspan="4">cooling tunnel 2</td><td>29</td></tr>
<tr><td>22</td><td>28</td></tr>
<tr><td>23</td><td>27</td></tr>
<tr><td>24</td><td>26</td></tr>
<tr><td>25</td><td colspan="3">cross transport</td></tr>
</table>

Figure 3.5: Numbered stations per mold of the praline forming plant ubi® PN-Y; molds move from position 1 to 49

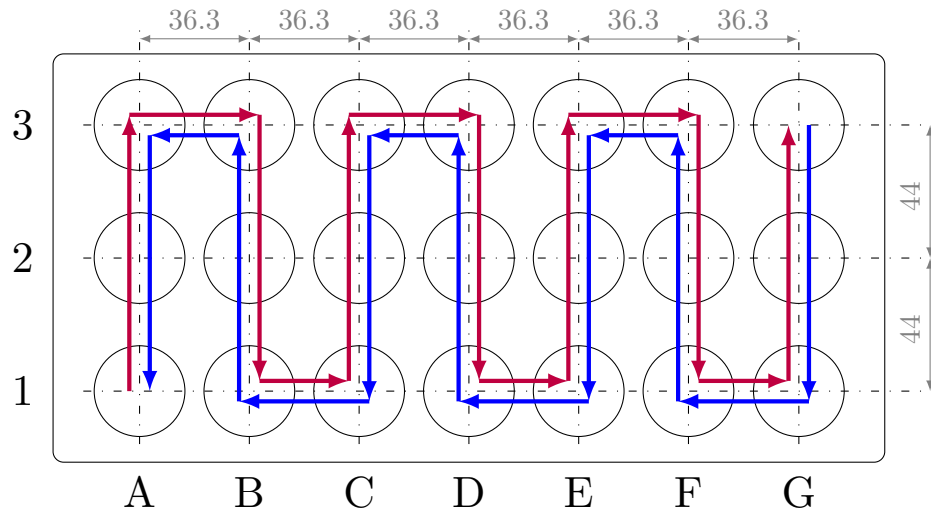


Figure 3.6: Molds with dimension of 275 mm × 135 mm × 24 mm (length × width × height) and the two lanes for mold filling from A1 to G3 (red) and in reverse direction G3 to A1 (blue)

stepless and independently adjustable. The cooled plunge, whose shape is adjusted to the used molds is cooled by a Peltier element.
The plant includes three cooling tunnels. The first one is used to cool the cold formed shells and give time for stabilization. Afterwards shells can be filled and sealed with sink head 2. This one-shot sink head can also be used independently from the before mentioned facilities to produce one-shot pralines, as described in chap. 2.5.3. Just as the first sink head 1, it moves towards the single cavities and it is followed by vibrator 2. Subsequently the samples are cooled further in the second and third cooling tunnel, which are connected by the cross transport.
The system was operated using a touch screen and system control by Julius GmbH (Marienheide, Germany). In the following tab. 3.3 the parameters and their meaning are shown. Within the software for the control system, the plant is divided in several units: sink head 1, cooled plunge, sink head 2, facility 1 and facility 2. In each unit parameters can be set and the elements can be switched on or off. The system is operated with its own parameters, which will be called machine relative units (m.r.u.).

Additionally to the settings at the plant, external coolers and a heater were used. A Systemcooler SC 1.7 VS ers® (Energie- und Kältetechnik GmbH, Strassenhaus, Germany), which was set to 4.0 °C was used for the cooling tunnels. For counter-cooling of the Peltier element a Systemcooler SC 1.7 V ers® (Energie- und Kältetechnik GmbH, Strassenhaus, Germany) at 18.0 °C was used. The heater was a water bath teco cs 90e (Gesellschaft Wärme Kältetechnik mbH, Kierspe, Germany) set at temperatures from 33.0 °C to 35.0 °C. It was used to heat the storage tank and the sink heads to keep the chocolate in molten and tempered state. The TI was measured intermittently during production. Therefore a tempermeter cup was filled with chocolate from the dosing

Table 3.3: Used parameters in machine relative units (m.r.u.) and their meaning for praline production using the praline plant ubi® PN-Y (ubitec GmbH, Bergneustadt, Germany)

unit	parameter	m.r.u.	meaning
sink head 1	dosing stroke	25	dosing quantity of sink head 1
	dosing speed	95	speed of dosing
	back suction stroke	8	back suction stroke after dosing
	excavation stroke	80	nozzle lifting distance during dosing
	excavation stroke speed	60	nozzle lifting speed during dosing
cooled plunge	plunge stroke	250	mold lifting distance
	speed	50	mold lifting speed
	cooling on	16	switch on of the Peltier element when the named cavity gets dosed
sink head 2	dosing stroke filling	40	dosing quantity of the filling
	dosing speed	85	speed at which filling and shell are dosed
	dosing stroke shell	0	dosing quantity of shell
	back suction stroke	15	back suction stroke after dosing of shell and filling
facility 1	mold preheater		switch On/Off
	sink head 1		switch On/Off
	vibrator 1		switch On/Off
	cooled plunge		switch On/Off
	vibrating roller		switch On/Off
	sink head 2		switch On/Off
	vibrator 2		switch On/Off
facility 2	vibrating time 1	61	vibrating time in tenths of a second of vibrator 1
	vibration start 2	1	vibration start of vibrator 2 at the named cavity
	vibration stop 2	5	vibration stop vibrator 2 at the named cavity
	heater on	1	start of mold preheater at cavity
	heater off	8	stop of mold preheater at cavity
	empty run w/o molding		switch On/Off; form transportation without dosing

stroke of sink head 1.
The praline plant can also be used to produce empty shells and fill them in a second round. The advantage of using the praline plant is an equal filling height. However, the nougat temperature has to be continuously controlled, since the temperature for the two storage tanks and sink heads (or filling and chocolate) can not be controlled individually. The nougat was melted at temperatures above 30 °C and cooled to (29.0 ± 0.5) °C by adding solid nougat.

3.5.3 Simulation of unintended mixing during production

For some pralines, hazelnut oil was added to the shell chocolate. The aim was to simulate mixing of chocolate and filling during production, as described in chap. 2.6.2.2. In this case the chocolate and hazelnut oil were first mixed and tempered afterwards. Concentrations were 5 mg/g, 10 mg/g, 20 mg/g, 30 mg/g, 40 mg/g and 50 mg/g hazelnut oil in chocolate. This chocolate-hazelnut-oil-mixture was used to produce cold stamped praline shells as described in chap. 3.5.2. The shells passed all three cooling tunnels and were stored subsequently at 18 °C over night before they were filled the next day. For filling, the shells remained in the molds which were fed into the praline plant. The mold preheater was switched off and at sink head 1 nougat was filled into the shells. Then they were cooled in the first cooling tunnel and a chocolate sealing was deposited at sink head 2. After passing cooling tunnel 2 and 3, the pralines were demolded and stored at 18 °C, 20 °C and 23 °C.

3.5.4 Pralines with post treatments

Post treatment of pralines is performed to reduce fat bloom in industrial production. Some post treatments were investigated to identify their efficacy and the mechanism of action. The aim was to investigate the usefulness of such treatments and to learn more about their impact on the underlying mechanisms of migration and crystallization.

3.5.4.1 Identification of optimum post treatment conditions via DSC

To identify post treatment conditions for pralines as described by Juul 2010, the production process was simulated using DSC. Therefore tempered chocolate was placed in an DSC aluminum pan. To ensure that the degree of crystallization was not changed, the aluminum pan was kept at 29 °C. Samples were measured using the following time-temperature protocol. For stabilization, the sample was held at 29 °C for 0.2 min followed by simulation of the cold plunge: The sample was cooled to −10 °C at 100 °C/min and held for 0.1 min at this temperature. Subsequently the cooling tunnel was simulated by heating to 18 °C at 100 °C/min and holding for 5 min until warm post tempering process was simulated by heating the sample to different temperatures T_{post} at 100 °C/min and holding for 3 min at this temperature. To evaluate the post tempering treatment a melting curve was added starting from T_{post} and heating to 45 °C at 3 °C/min and holding for 1 min. Investigated temperatures T_{post} were 25 °C, 28 °C, 29 °C, 30 °C, 32 °C

and 33 °C. As a reference, a sample without post treatment was measured for which T_{post} was set to 18 °C.

3.5.4.2 Application of post treatment on praline shells

According to the results of process simulation via DSC, the praline shells were treated as follows. The praline shells were taken from the praline plant after the first cooling tunnel at position 19, shown in fig. 3.5 and in fig. 3.7. They were placed in a conditioning cabinet Incucell LSIS-B2VI IC222 (MMM Group, Planegg, Germany) at the determined temperature T_{post} and remained there for 15 min. The time was extended relative to DSC measurement to ensure equal temperature throughout the whole shell. As a reference a shell without post treatment was prepared by placing the shell in a conditioning chamber at 18 °C for the according time.

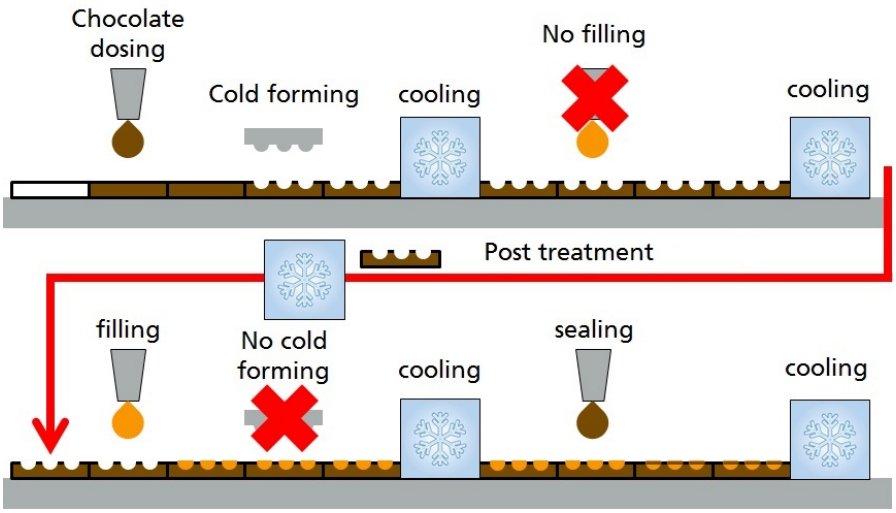


Figure 3.7: schematic drawing of shell manufacturing with post treatment

After post treatment samples were stored for 2 h at 18 °C and subsequently filled with nougat, using a disposable syringe with a volume of 20 mL (Henke-Sass, Wolf GmbH, Tuttlingen, Germany). An amount of 4 mL nougat was filled in each shell. After filling, the samples were shaken to remove air bubbles and to evenly spread the nougat followed by sealing with a layer of tempered chocolate.
After production sample pralines were stored at 18 °C for 24 h, demolded and further stored at 18 °C, 20 °C and 23 °C.

3.5.5 Praline storage tests at isothermal and cycled temperature

Different storage tests are used to accelerate fat bloom formation (see chap. 2.6.2.3). To identify the optimum tests for this study, different storage conditions were used and fat bloom formation was observed.

The praline shells were produced as described in chap. 3.5.2. After the first cooling tunnel, the shells were directly filled with nougat using sink head 2 without a chocolate sealing. After production pralines were stored for 20 h at 18 °C and subsequently storage tests were started. Pralines were stored at varying conditions and analyzed over time. Main analyzed parameters were filling migration, polymorphic transition and fat bloom development as described in chap. 3.6. All samples were stored isothermally at different constant temperatures, namely 18.0 °C, 20.0 °C, 23.0 °C, 25.5 °C and 27.0 °C and at cycling temperatures for different times as can be seen in tab. 3.4.

Table 3.4: Conditions for 40 d of cycling temperature storage

start temperature	cycling temperature	frequency
18.0	25.5	three times a week
25.5	18.0	three times a week
18.0	25.5	weekly
25.5	18.0	weekly

The storage temperature was changed by manually putting the samples into another conditioning cabinet or chamber. Samples were stored in two conditioning cabinets Incucell LSIS-B2VI IC222 (MMM Group, Planegg, Germany) set to 25.5 °C and 27.0 °C and different conditioning chambers (Viessmann Allendorf (Eder), Germany and York Deutschland GmbH, Mannheim, Germany) at 18.0 °C, 20.0 °C and 23.0 °C. Random control of temperatures and humidity were performed using a data logger Easylog EL-USB-2+ (Lascar electronics, Salisbury, United Kingdom). Temperature was constant ±0.5 °C and relative humidity fluctuated between 24 %rH to 34 %rH.

3.6 General instrumental analysis

3.6.1 Fat analysis

FA and TAG composition were determined as described by Rothkopf and Danzl 2015. The used chemicals are listed in tab. 3.5.

3.6.1.1 Fat extraction

Samples were weighed in 2 mL Eppendorf tubes (Eppendorf AG, Hamburg, Germany). Sample weight was adjusted to fat content, so that each sample contained about 10 mg fat. Until further processing, samples were stored at −20 °C. Samples were mixed with 1 mL n-hexan, including internal standard for GC measurements. To completely dissolve the samples they were shaken at 25 °C with 1300/min for 15 min using a Thermomixer Comfort (Eppendorf AG, Hamburg, Germany) followed by centrifugation for 5 min at 800/min with a WiseSpin® CF-10 (LENNOX, Dublin, Ireland). The supernatant was transferred in crimp-top vials ND 11 with 1.5 mL volume (Th. Geyer GmbH & Co. KG, Renningen, Germany) and purged with nitrogen at 40 °C to replace the solvent.

3.6.1.2 Fatty acid determination via GC-FID

The FA composition was determined via GC-flame ionization detector (FID). About 5 mg to 9 mg of sample was dissolved in 1 mL tert-butylmethylether and filtrated using a PTFE-membrane. The filtered samples were put into vials which were filled with 0.2 mL to 0.25 mL of 0.2 mol/L methanolic trimethylsulfoniumhydroxide (TMSH) solution for esterification. Vials were sealed with crimp caps (Th. Geyer GmbH & Co. KG, Renningen, Germany) and heated to 100 °C for 15 min. After cool-down, measurement was started using a GC-System 7890A (Agilent Technologies, Santa Clara, USA), with OpenLAB CDS ChemStation GC Drivers (Agilent Technologies, Santa Clara, USA) and a Chrompack FFAP-CB column (50 m × 0.45 mm × 0.3 mm). Injector temperature was 250 °C with 1:20 split and 1 µL injection volume. Carrier gas was hydrogen and oven temperature was held at 200 °C for 10 min, heated to 250 °C with 30 °C/min and held for another 10 min for cocoa butter and hazelnut oil. In case of butterfat and coconut fat, oven temperature was held at 40 °C for 2 min, heated to 250 °C with 30 °C/min and held for 10 min. Detector temperature was 260 °C and gas flow was 40 mL/min hydrogen, 450 mL/min air and 30 mL/min nitrogen make-up. For quantification, two internal standards were used. Tridecanoic or heptadecanoic acid was used for samples containing hazelnut oil and valeric acid for samples with short-chained fatty acids (less than ten carbon atoms), such as butyric acid. Measurements were carried out in triplicate.

3.6.1.3 Triacylglyceride determination via RP-HPLC-ELSD

The TAG composition was determined using a high performance liquid chromatography (HPLC) with Solvent rack SOR-100, HPLC pump P680, automated sample injector

Table 3.5: Used chemicals for fat analysis by GC and HPLC

chemical	concentration [n/n] − %	additional information	supplier
acetonitrile	99.9	gradient grade for HPLC	CHEMSolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany
ethanol	99	for analysis	CHEMSolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany
methanol	99.85	gradient grade for HPLC	CHEMSolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany
n-hexan	99	for analysis	CHEMSolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany
tert-butylmethylether	99.8	gradient grade for HPLC	CHEMSolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany
heptadecanoic acid	98		Alfa Aesar, Thermo Fisher GmbH, Karlsruhe, Germany
hydrochloric acid	35 to 38	for analysis	CHEMSolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany
phenolphthalein		pH 8.2 to 9.8	MERCK KGaA, Darmstadt, Germany
sodium hydroxide	99		MERCK KGaA, Darmstadt, Germany
tridecanoic acid	98		SIGMA-ALDRICH®, St. Louis, USA
trimethylsulfonium iodide	98		SIGMA-ALDRICH®, St. Louis, USA
valeric acid	99		SIGMA-ALDRICH®, St. Louis, USA

ASI-100 and thermostated column compartment TCC-100 (Dionex Softron GmbH, Germering, Germany). An amount of 10 mg of each sample was weighed into a 10 mL volumetric flask. The flask was filled to the mark with the solvent, which consisted of 0.6 L/L acetonitrile and 0.4 L/L tert-butylmethylether. A volume of 2 µL to 5 µL was injected and a flow rate of 0.5 mL/min was used. Oven temperature was 18 °C. As detector a LT-evaporative light scattering detector (ELSD) SEDEX 85 (Sedere S.A., Olivet, France) was used at 4 bar and 40 °C. A Security Guard Cartridge C18 4×3 mm ID (Phenomenex, Aschaffenburg, Germany) was used in front of the capillary column Nucleosil ET 250 cm × 4 mm ID 120-3 µm C18 (Macherey Nagel, Düren, Germany) with 125 mm length and 4 mm diameter.

3.6.2 NMR - nuclear magnetic resonance

The SFC, the amount of solidified fat, is influenced by many factors, such as the sample composition including the amount of fat and its composition, as well as the thermal history. Therefore it can be used as an indicator for fat composition in samples with same thermal treatment or to see differences in treatment of samples with same composition. A minispec mq 20 TD-NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) was used to detect the SFC. Two methods for SFC detection by NMR exist, the direct and the indirect. The determination of the SFC is explained using an exemplary decreasing signal of a fat sample after a 90° high frequency impulse, shown in fig. 3.8.

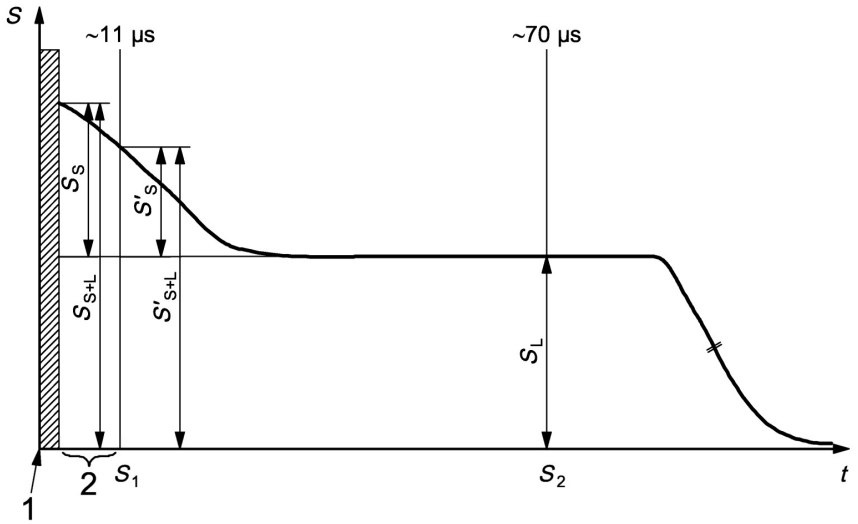


Figure 3.8: Decreasing signal of a fat samples after a 90° high frequency impulse (DIN EN ISO 2010a)

In the graph S represents the decreasing signal, t the time, 1 is the high frequency impulse and 2 the dead time, S_S the signal of the solid phase and S_{S+L} of the solid and the liquid phase at time $t = 0$. Due to the dead time shown as hatched area, the signal

can just be detected after some micro seconds. Therefore the signal S_1 after about 11 µs is used. It equals S'_{S+L}, which includes the signal of the solid and the liquid phase at that time. S'_S is the signal of the solid phase at that time. A second signal S_2 after 70 µs is recorded, which equals S_L, the signal of the liquid phase at that time. The signal for the solid phase is already zero due to the faster signal decay in the solid phase.
For the **direct method** (DIN EN ISO 2010a) only one measurement needs to be done. The ratio of the signal S_{S+L}, which includes the signal of the solid and the liquid phase, and S_L, which includes the signal of the liquid phase is used to calculate the direct SFC_d. Due to the dead time, the signal of the solid and liquid phase S_{S+L} can not be detected directly after the high frequency impulse. Therefore an extrapolation factor f is used to calculate S_{S+L} using S'_{S+L} resulting in eq. 3.6.1.

$$SFC_d = \frac{f(S'_{S+L} - S_L)}{f(S'_{S+L} - S_L) + S_L} \tag{3.6.1}$$

The direct method results in an approximate value because of the f-factor. A linear extrapolation is assumed to calculate the signal at $t = 0$, but the signal shows a non-linear decrease. Additionally, f depends on the molecular mobility, e.g. temperature, polymorphism and crystal size and its temperature dependency is also due to the dilatation of the liquid phase. Typical values for f are given in DIN EN ISO 2010a and range from 1.10 to 1.30; 1.40 to 1.50 and 1.60 to 2.00 for α, β' and β, respectively. Another influencing value is the solid phase in samples with non-fat solids. Thus, the direct method was only used for pure fat samples.
For all samples with particles, the **indirect method** must be used. For the indirect method (DIN EN ISO 2010b), only the signal S_2 of the liquid phase of the sample after about 70 µs is measured. The signal of the liquid phase of a fully molten sample is measured at the temperature T_{melt} and the signal of the liquid phase of a crystallized sample is measured at the temperature T_{crys}. Additionally, the signal of the liquid phase of a reference is recorded at the same temperatures. Oils that are liquid at T_{crys} were used as reference, namely sunflower, rapeseed and hazelnut oil. The values of the reference are needed for temperature correction. From these four values, the SFC_{ind} can be calculated using eq. 3.6.2.

$$SFC_{ind} = 1 - \frac{S_{2,crys} \cdot S_{2,ref,melt}}{S_{2,melt} \cdot S_{2,ref,crys}} \tag{3.6.2}$$

Both methods can be used to either determine the SFC at a certain temperature or to evaluate crystallization (Rothkopf and Danzl 2015). For both direct and indirect measurement, solid samples were chopped before they were placed in glass tubes and compressed inside them. Molten samples were directly filled into the glass tubes. The tubes were sealed with parafilm®. Filling height varied between 30 mm to 50 mm for the direct method and 8 mm to 10 mm for the indirect method.

3.6.2.1 Sample preparation for SFC determination with structure preservation

To observe changes caused by storage, samples were prepared without changing the initial crystal structure. For this purpose solid and stored samples were used. Samples were cut in small pieces and placed in a glass tube. They were subsequently stored at 20 °C for 24 h and measured with f-factor 1.8, four repetitions and 2 s recycle delay.

3.6.2.2 Standardized sample preparation with β-stabilization for SFC determination

A standardized crystallization was used to prepare NMR samples for SFC determination, which allows to detect differences in the composition of samples. The aim of the procedure is to erase the thermal history and to achieve a stable β crystal polymorph, which ensures a good reproducibility of the measurement (DIN EN ISO 2010a; DIN EN ISO 2010b).
Samples were subject to the following time-temperature protocol: melting for (30±2) min at 70 °C to melt any remaining crystals was followed by cooling for (90 ± 2) min at 0 °C in an ice bath to start crystallization. For β-stabilization, samples were stored for (40.0 ± 0.5) h at 26 °C to melt instable crystals and allow further crystallization of stable crystals. Afterwards a last crystallization step for (90 ± 2) min at 0 °C in an ice bath was performed. Samples were stabilized at the measuring temperature (T_{crys} =20 °C) for (60 ± 2) min and subsequently measured. The f-factor was set to 1.8, with four repetitions and 2 s recycle delay.

3.6.2.3 Dynamic, quasi-isothermal crystallization measurement

Crystallization kinetics can also be detected using NMR. For this purpose samples were heated at 70 °C for (30 ± 2) min to melt any remaining crystals. Then they are placed in the probe-head, which is kept at 19 °C. A SFC measurement is performed every 30 s with three repetitions and 1.5 s recycle delay. Similar procedures are described by Padar, Jeelani, et al. 2008; Bootello et al. 2013.
The start point had to be set for evaluation. Therefore the indirect SFC (see eq. 3.6.2) was determined. Instead of a reference the sample was measured at several temperatures above the melting point. The samples were melted for 30 min at 70 °C and cooled for 1 h to 60 °C, 50 °C and 40 °C in decreasing order. Each temperature step was followed by a SFC measurement, which was used to extrapolate the temperature corrected value $S_{2,melt,corr}$ of a molten sample at 20 °C. This simplifies eq. 3.6.2 to eq. 3.6.3.

$$SFC_{ind,corr} = 1 - \frac{S_{2,crys}}{S_{2,melt,corr}} \tag{3.6.3}$$

To evaluate fast, dynamic, quasi-isothermal NMR crystallization measurements, the calculated $SFC_{ind,corr}$ is plotted over time, as can be seen in fig. 3.9. In the beginning the curve decreases due to temperature adaptation from 70 °C to 19 °C of the sample. The time after this adaption was determined as the time, when slope > 0 is true and this time was used as starting time.

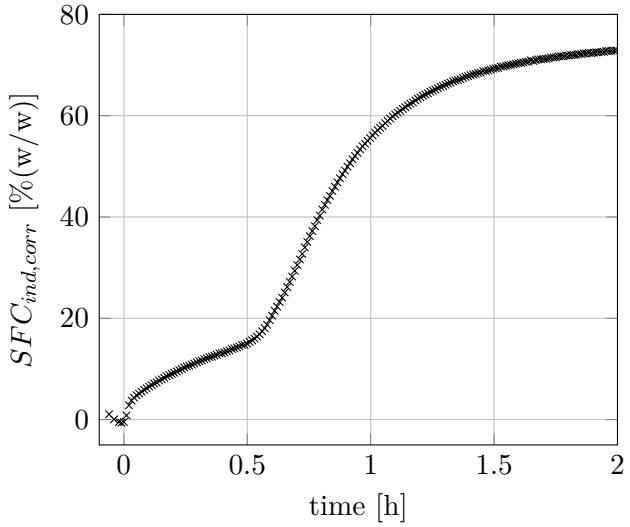


Figure 3.9: Curve progression of $SFC_{ind,corr}$ at 19 °C of cocoa butter CB 02 plotted over time

3.6.3 DSC - differential scanning calorimetry

A DSC 8500 with Intracooler 2 (Perkin Elmer LAS GmbH, Rodgau-Jügesheim, Germany) was used for calorimetric measurements. An empty aluminum pan was used as reference. The thermal analysis system was purged with nitrogen. Thermograms were analyzed with Pyris Software (Version 10.1.0.0412, Perkin Elmer, Rodgau-Jügesheim, Germany).

3.6.3.1 Melting curves

Melting curves were used to determine the polymorphic form of the cocoa butter crystals in the sample, which is indicated by the peak maximum temperature. The sample weight was about (10±2) mg. The time-temperature protocol starts at 20 °C and increases with 3 °C/min up to 45 °C. In fig. 3.10 an exemplary melting curve is shown.

3.6.3.2 Cooling curves and isothermal crystallization

Both, cooling curves and isothermal crystallization are used to investigate the crystallization behavior of cocoa butter and chocolate. The sample weight was (18 ± 2) mg and all samples were heated to 70 °C for 5 min prior to analysis to melt any remaining crystals and to erase the thermal history.
For isothermal measurements the samples were cooled from 70 °C to the crystallization temperature of 19 °C at 20 °C/min and held for 120 min until crystallization was finished. An exemplary isothermal DSC measurement and the peak minimum time, which was used for evaluation, are shown in fig. 3.11.

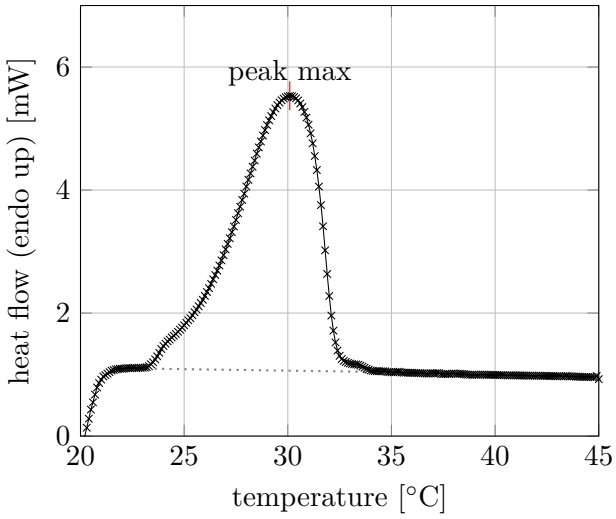


Figure 3.10: Heat flow of cocoa butter CB 02 recorded by DSC during melting at 3 °C/min from 20 °C to 45 °C with peak maximum, which was used for evaluation, and baseline (dotted gray)

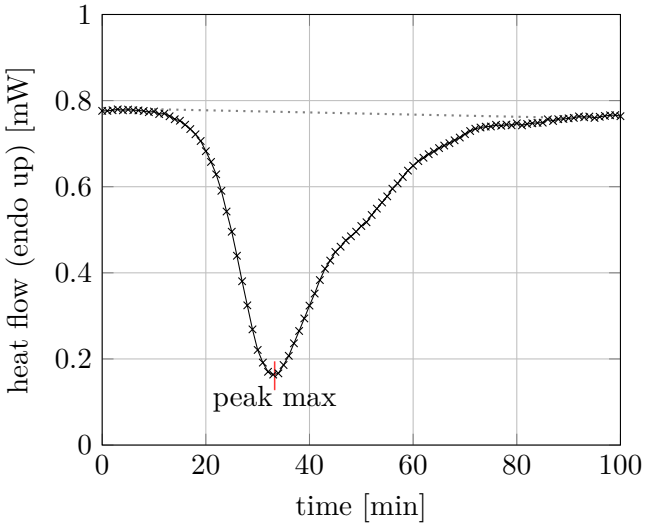


Figure 3.11: Heat flow of CB 02 recorded by DSC during isothermal crystallization at 19 °C, peak maximum time and baseline (dotted gray)

Another measuring method using DSC are cooling curves, where the temperature is continuously decreased over time. For cooling curves the samples were melted as previously described for 5 min at 70 °C and cooled from 70 °C to 40 °C at 10 °C/min and further to −10 °C with a cooling rate of 2 °C/min. An exemplary curve is shown in fig. 3.12. The peak minimum temperature was taken directly from the measured heat flow over time and used for evaluation.

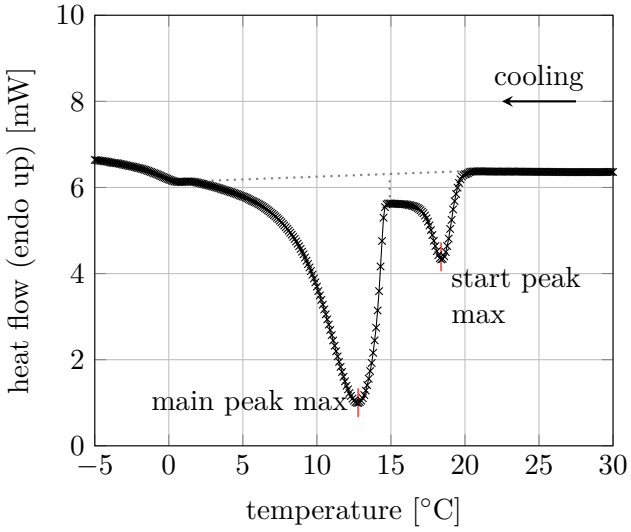


Figure 3.12: Heat flow of CB 02 recorded by DSC during cooling crystallization with peak maximum and baseline (dotted gray)

3.6.4 Free fat

The amount of free fat is determined using a centrifuge. Samples were melted at 45 °C and about 7.0 g were weighed into a 50 mL centrifuge tube with screw cap (Thermo Fisher Scientific Inc., Waltham, USA) and further heated for 1 h at 60 °C. A centrifuge Sigma 3 K 30 with angle rotor for 8 × 50 mL (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) was used at 40 °C for 60 min with 13 650/min revolution, which equals 20 000 g RCF.
After centrifuge, the samples were set upside down in a climate chamber at 60 °C for 1 h to remove the supernatant fat. Subsequent, samples were cooled and weighed using a XS205 DualRange (Mettler Toledo AG, Gießen, Germany). The amount of free fat can be calculated as follows:

$$free\ fat = \frac{m_{int} - m_{res}}{m_{int} * fat\ content} \tag{3.6.4}$$

The initial weight m_{int} is the mass before centrifuge and the residual mass m_{res} is the mass after the supernatant fat was drained off.

3.6.5 Particle size distribution

For particle size distribution measurement a Malvern® Morphologi G3 (Malvern Instruments, Malvern, Great Britain) was used. Samples were melted at 45 °C and about 40 mg were dispersed in 25 mL vegetable oil using a magnetic stirrer. The dispersed samples were filled in a wet cell using a disposable syringe. After letting the particles sediment for about 30 min, the measurement was started with 20× magnification, 60 % light intensity, threshold 140 to 145 and smoothing of 40 points. After counting of 200 000 particles, the measurement stopped. For evaluation, incompletely captured particles were excluded by using circularity as indicator.

3.6.6 BET-surface

The surface area of the samples were determined by krypton adsorption-desorption isotherms at −196 °C (77 K) using a Quantachrome Autosorb iQ automated gas sorption analyzer. Prior to the analysis, the powder samples were outgassed under vacuum at 50 °C for 5 h. The specific surface area was calculated by using the Brunauer Emmett Teller (BET) equation. All calculations were performed by an ASiQwin (Version 5.0) program developed by Quantachrome Instrument (Quantachrome Instruments 2020).

3.6.7 Fat bloom evaluation

Fat bloom can be evaluated by either using a trained or customer panel or color and gloss measurement (Briones and Aguilera 2005). Using color or gloss measurements allows to create a number signifying the amount of whiteness on the chocolate surface.
Fat bloom was evaluated using the DigiEye Imaging System V2.60. The system consisted of a digital camera Nikon D90 placed on a light cabinet with a lamp VeriVide D65 "Artificial Daylight". A daily calibration was performed using a digitizer chart (all components from VeriVide Ltd., Leicester, United Kingdom).
Samples were photographed on blue ground with 0.2 s exposure time, ISO sensitivity ISO-200, f-number F/8, focal width 35 mm and lighting set to diffuse.
For evaluation a shape (round or rectangle) was chosen according to the sample dimensions. The area was chosen smaller than the size of the sample, since sample edges sometimes behaved different then the main area. The L*, a* and b* values were determined by the DigiEye Software and were used to calculate the Whiteness Index (WI) (Briones and Aguilera 2005).

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \tag{3.6.5}$$

3.6.8 Statistics and curve analysis

Statistics were performed using SigmaPlot 12.5 (Systat Software Inc.) and OriginLab. SigmaPlot was used for ANOVA and according calculations, such as normality and equal variance test. OriginLab was used for calculation of curve mean values with linear interpolation including standard deviation.

4 Results and discussion

4.1 Filling fats and oils in cocoa butter and chocolate

During chocolate production, filling fats and oils can unintentionally get into the chocolate (Rothkopf, Danzl, and Ziegleder 2016). These are named *initial oils and fats* and they might affect crystallization and the resistance against migration. Therefore the effect of fats and oils on cocoa butter crystallization was studied.

4.1.1 Fat analysis

The composition of fatty acid (FA) and resulting triacylglyceride (TAG) in all used filling fats and oils as well as in cocoa butter and chocolate samples was determined.
The FA composition of the added fats was determined as described in chap. 3.6.1 and results are given in tab. 4.1 for short chained FA and in tab. 4.2 for long chained FA.

Table 4.1: Fatty acid composition of coconut fat and butterfat from C4:0 to C14:0 in mg/g for three replicates as mean ± standard deviation

fatty acid	butyric C 4:0	caproic C 6:0	caprylic C 8:0	capric C 10:0	lauric C 12:0	myristic C 14:0
coconut fat		7±1	82±7	62±4	471±20	182±5
butterfat	31±3	20±1	13±1	30±2	36±3	112±10

FA with a short carbon chain length of 4 to 14 C-atoms were only found in coconut fat and butterfat in detectable quantities. Butyric acid (C4:0) was only found in butterfat and lauric acid mainly in coconut fat. Myristic acid was noticeable in both fats. Butterfat and coconut fat show clear differences in FA composition.
The long chained FA, shown in tab. 4.2, of coconut fat and butterfat differ clearly from each other and from the investigated oils. The investigated oils contain similar amounts of palmitic and stearic acid, but differ clearly in oleic and linoleic acid content. Additionally, (121 ± 1) mg/g of linolenic acid (C18:3) were found in walnut oil.

Table 4.2: Fatty acid composition in mg/g for three replicates as mean ± standard deviation and sum of means from C4:0 to C18:2

sample	palmitic C16:0	stearic C18:0	oleic C18:1	linoleic C18:2	sum C4:0 to C18:2
almond oil	57±1	17±1	665±3	252±1	991
butterfat	285±26	92±9	223±13	21±2	863
coconut fat	77±5	58±4	30±2		969
hazelnut oil	55±1	21±1	809±3	110±1	995
olive oil	102±6	27±1	770±16	66±11	965
walnut oil	68±1	28±1	171±1	607±2	874
CB 01	252±1	353±1	338±1	30±1	973
CB 02	255±1	360±1	334±1	27±1	976
CB 03	253±1	363±1	331±1	28±1	975
CB 04	259±1	356±1	333±1	28±1	976
dark chocolate	240±10	348±18	316±15	29±1	933

The long chained FA, palmitic, stearic, oleic and linoleic acid of dark chocolate and cocoa butter vary only slightly. The resulting TAG are shown in tab. 4.3 and 4.4.

Table 4.3: Main TAG of cocoa butter in mg/g based on total quantified TAG, three replicates as mean ± standard deviation (P:palmitic, S:stearic, O:oleic)

	POP	POS	SOS	sum
CB 01	168±1	420±4	273±4	861
CB 02	172±1	429±1	278±2	879
CB 03	167±1	431±1	281±1	879
CB 04	178±1	429±3	269±3	876

Due to the high number of possible TAG resulting from the FA found in the filling fats and oils, only the main TAG of the cocoa butter samples are given.The main TAG in all investigated cocoa butter samples are POP, POS and SOS. The minor TAG which could be identified were PLP, POO, SOO and SSS. Their amount varied only slightly.

4.1.2 Crystallization of cocoa butter mixed with oils and fats

Crystallization of cocoa butter depends on the TAG composition. To investigate its influence, cocoa butter was mixed with vegetable oils and edible fats. In chocolate the presence of particles causes heterogeneous nucleation and the effect of TAG addition might vanish. Therefore, cocoa butter was used instead of chocolate in this study.
Cocoa butter CB 01 was mixed with vegetable oils with differing TAG composition as well as coconut fat and butterfat, which are partly solid at room temperature compared

Table 4.4: Minor TAG of cocoa butter in mg/g based on total quantified TAG, three replicates as mean ± standard deviation (P:palmitic, S:stearic, O:oleic, L:linoleic)

	PLP	POO	SOO	SSS
CB 01	19±1	55±1	52±1	13±1
CB 02	18±1	48±1	41±1	14±1
CB 03	18±1	49±1	41±1	12±1
CB 04	19±1	50±1	42±1	13±1

to the vegetable oils. Adding edible fats to cocoa butter can cause co-crystallization and formation of crystallite composition. Butterfat is included in big amounts in milk chocolate, while coconut fat can be washed of from pastries during the enrobing process and is known to be incompatible to cocoa butter due to its high amount of lauric acid.

4.1.2.1 Crystallization of cocoa butter mixed with oils measured by NMR

Crystallization was recorded by fast, dynamic, quasi-isothermal nuclear magnetic resonance (NMR) measurements at 19 °C, as described in chap. 3.6.2.3. The influence of almond, hazelnut, olive and walnut oil addition was investigated at concentrations of 50 mg/g, 100 mg/g and 150 mg/g oil addition based on the weight of cocoa butter and is shown in fig. 4.1, 4.2 and 4.3 in comparison to pure cocoa butter CB 01 as a reference.

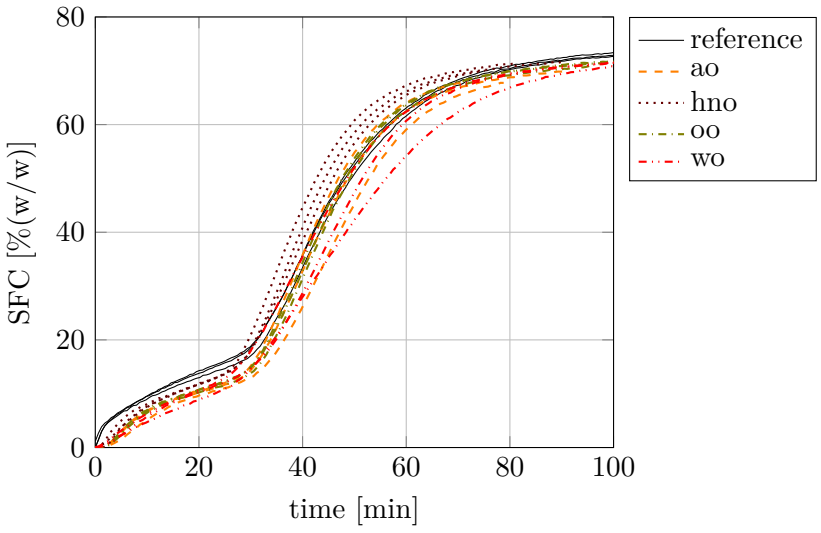


Figure 4.1: NMR fast, dynamic, quasi-isothermal crystallization at 19 °C of cocoa butter CB 01 (reference) and mixed with 50 mg/g almond (ao), hazelnut (hno), olive (oo) and walnut oil (wo) based on cocoa butter weight from three measurements each

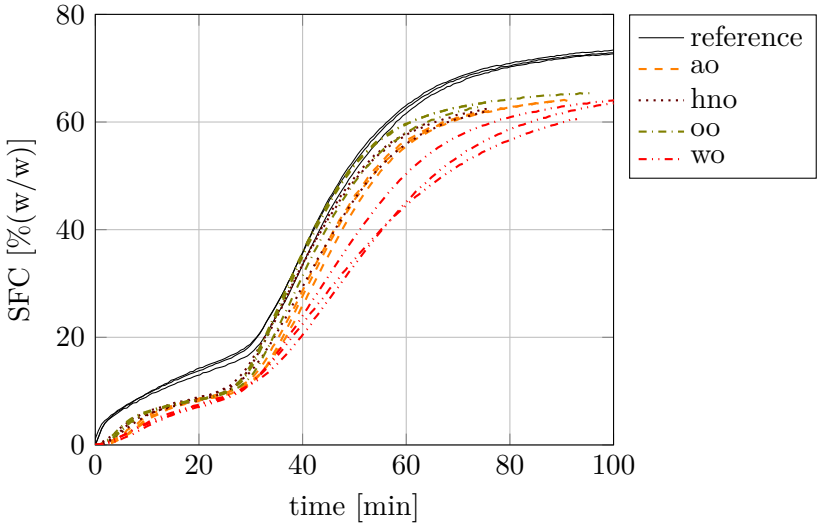


Figure 4.2: NMR fast, dynamic, quasi-isothermal crystallization at 19 °C of cocoa butter CB 01 (reference) and mixed with 100 mg/g almond (ao), hazelnut (hno), olive (oo) and walnut oil (wo) based on cocoa butter weight from three measurements each

Plotting solid fat content (SFC) over time during fast, dynamic, quasi-isothermal NMR crystallization measurements at 19 °C shows a sigmoid curve progression for pure cocoa butter CB 01 and for mixtures with different amounts of almond, hazelnut, olive and walnut oil. During the first 30 min of the crystallization process the oil addition leads to a lower SFC and a curve progression with less slope for all oils compared to the pure cocoa butter reference CB 01 . 30 min after the start of the crystallization a kink can be seen, which appears at almost the same time for samples with almond, hazelnut and walnut oil addition. Adding olive oil to the cocoa butter leads to an earlier appearance of the kink. The further curve progression changes depending on the type and amount of oil. Walnut oil reduced the SFC increase the most for all concentrations. At 50 mg/g oil addition, hazelnut oil increases the SFC increase compared to the other oils and to pure cocoa butter. Curve progression after the first kink of samples with almond oil and olive oil addition of 50 mg/g are comparable to pure cocoa butter. Adding 100 mg/g shows a different trend for the investigated oils. Compared to pure cocoa butter the SFC increase after the kink is reduced least by olive oil, followed by hazelnut oil, almond oil and strongest reduction is caused by walnut oil. This trend can also be observed when 150 mg/g oil are added. However, the difference between the crystallization curves of one sample increases with such a high oil amount for all types of oil. At the end of the measuring time the SFC of all samples progresses towards a similar final SFC value independent of the type of oil.

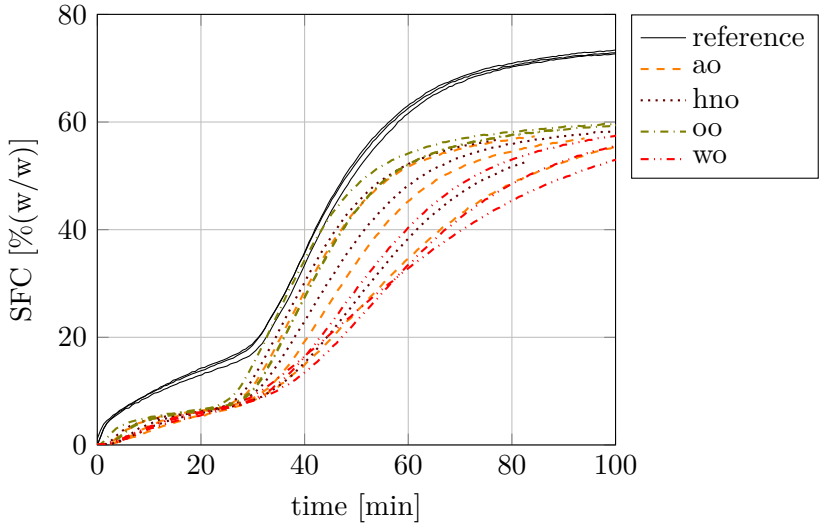


Figure 4.3: NMR fast, dynamic, quasi-isothermal crystallization at 19 °C of cocoa butter CB 01 (reference) and mixed with 150 mg/g almond (ao), hazelnut (hno), olive (oo) and walnut oil (wo) based on cocoa butter weight from three measurements each

4.1.2.2 Crystallization of cocoa butter mixed with oils measured by DSC

The isothermal crystallization of cocoa butter and vegetable oil mixtures was also recorded by differential scanning calorimetry (DSC) at 19 °C as described in chap. 3.6.3.2. The evaluated peak maximum time indicates the time for maximum crystallization rate and is shown in fig. 4.4.

The time to reach the peak maximum is longer for samples with added oils compared to pure cocoa butter. A longer time to reach the peak maximum indicates a retarded crystallization. However, the time extension depends on the type of oil. For oil addition of up to 100 mg/g the trend for almond oil, hazelnut oil and olive oil does not show significant differences. Walnut oil has the least retarding effect. At 150 mg/g almond oil addition, the peak maximum appears at an earlier time compared to samples with hazelnut oil and olive oil addition at the same concentration.
In addition to isothermal crystallization, cooling curves, as described in chap. 3.6.3.2 were recorded, where the samples were subject to a constant temporal temperature decrease. The results of the evaluated peak maximum temperatures are shown in fig. 4.5 during DSC cooling curves with a cooling rate of 2 °C/min from 40 °C to −10 °C.

The peak maximum temperature obtained via DSC cooling curves is reduced by oil addition. Mixtures with 150 mg/g walnut oil show a higher peak maximum temperature than samples with hazelnut oil. For the other oils and lower concentrations no clear trend can be observed.

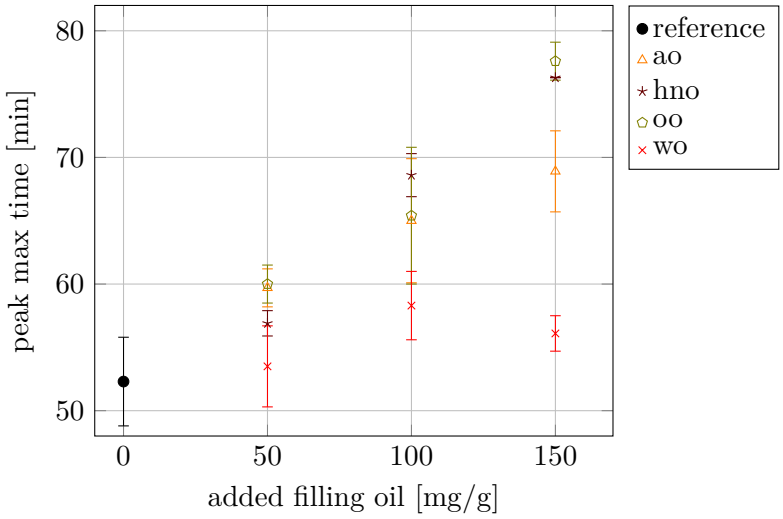


Figure 4.4: DSC peak maximum time of isothermally crystallized CB 01 as a reference and mixed with oils, namely almond (ao), hazelnut (hno), olive (oo) and walnut oil (wo) at 19 °C as mean of four measurements with standard deviation

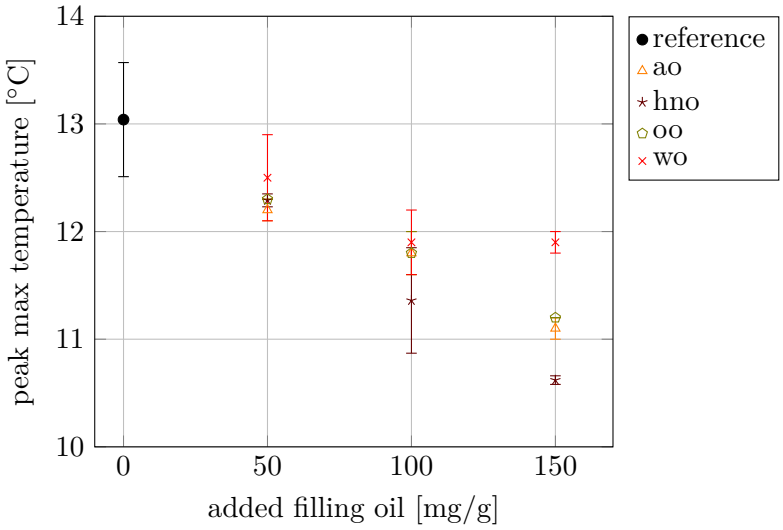


Figure 4.5: Peak maximum temperature of cooling curves measured via DSC with a cooling rate of 2 °C/min from 40 °C to −10 °C for cocoa butter CB 01 as a reference and mixed with oils, namely almond (ao), hazelnut (hno), olive (oo) and walnut oil (wo) as mean of four measurements with standard deviation

4.1.2.3 Solid fat content (SFC) of cocoa butter mixed with oils

To investigate the crystallization of different mixtures of TAG, CB 01 was mixed with vegetable oils, namely almond oil, hazelnut oil, olive oil and walnut oil. For SFC measurement the samples were prepared with β-stabilization and the measuring temperature was 20 °C as described in chap. 3.6.2.2. The SFC is shown in fig. 4.6 as well as the SFC as one would theoretically expect based purely on dilution. For the dilution it is assumed that both substances are completely miscible. The SFC of such a mixture of component A with SFC_A and component B with SFC_B and the mass fraction of $w_B = m_B/m_{total}$ can be calculated using eq. 4.1.1. This results in a straight line between the SFC of the pure substances. The SFC of an oil is 0 % per definition, which says that oils are totally liquid at room temperature.

$$SFC(w_B) = w_B \cdot SFC_B + (1 - w_B) \cdot SFC_A \tag{4.1.1}$$

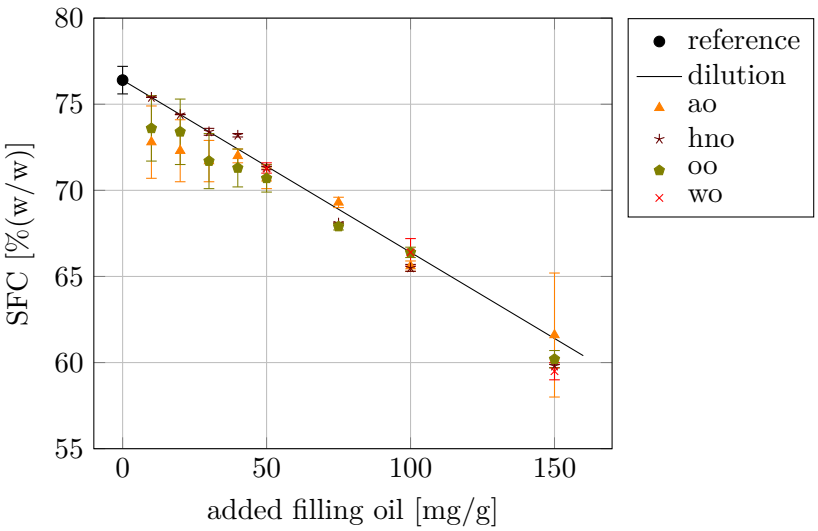


Figure 4.6: SFC at 20 °C of reference CB 01 mixed with different oils, namely almond oil (ao), hazelnut oil (hno), olive oil (oo) and walnut oil (wo) and the SFC of the mixtures which would be expected by dilution

The addition of filling oils to cocoa butter causes a decrease of SFC, which is comparable to the decrease, which would be expected by dilution. The type of oil has no impact.

4.1.2.4 Solid fat content (SFC) of cocoa butter mixed with fat

The SFC of cocoa butter mixed with coconut fat and butterfat is shown in fig. 4.7 as well as the SFC as one would theoretically expect based purely on dilution for each fat (see eq. 4.1.1). Samples were prepared with β-stabilization and the measuring temperature was 20 °C, as described in chap. 3.6.2.2.

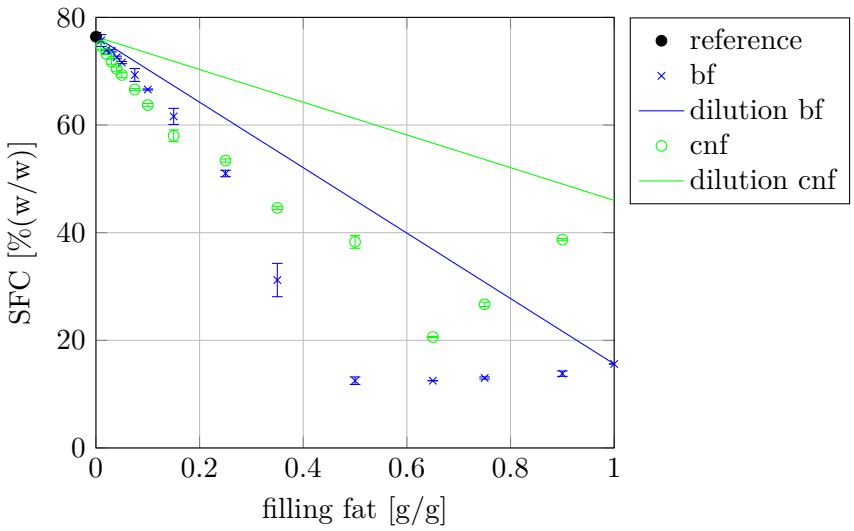


Figure 4.7: SFC at 20 °C of CB 01 as a reference mixed with increasing amounts of butterfat (bf) and coconut fat (cnf) and the SFC which would be expected by dilution

The addition of butterfat and coconut fat to cocoa butter leads to a SFC reduction. However, the measured SFC is clearly lower than expected by dilution, with the biggest difference at concentrations of 0.50 g/g to 0.65 g/g.

4.1.3 Discussion

Fat analysis The results of FA analysis showed similar values for all investigated fats and oils compared to literature. Differences might be caused by different measuring systems. Additionally the origin and variety is important.
The TAG composition was analyzed for cocoa butter samples only. The amount of the main TAG, namely POP, POS and SOS are comparable to literature. CB 01 showed higher amounts of SOO and POO compared to the other samples.
Crystal growth The main effects of vegetable oil addition on cocoa butter crystallization are observed for the phase of crystal growth. The main crystallization process includes crystal growth and crystal transition into more stable polymorphic forms. DSC cooling curves and isotherms show that vegetable oil addition causes lower temperatures of the peak maximum and longer times until the peak maximum is reached. Adding walnut oil has less effect on peak maximum temperature compared to the other oils. Fast, dynamic, quasi-isothermal NMR crystallization curves show the opposite trend. This might be caused by the different stages of crystal growth, which can be observed by NMR. In DSC isothermal and cooling crystallization measurements the main peak is evaluated. It refers to the main crystallization, which is characterized by crystal transition of less into more stable polymorphic forms of cocoa butter crystals. In the NMR measurements the crystal formation (first increase of the SFC) as well as the main crys-

tallization (second increase of SFC) can be observed and evaluated.
Fast, dynamic, quasi-isothermal NMR crystallization measurements curve progression was shown to be related to different stages of crystal growth (Marangoni and McGauley 2003). During nucleation not enough crystals are formed to be detected. The first SFC increase is caused by growth of crystals in an unstable polymorph such as α (Foubert, Dewettinck, Janssen, et al. 2006). The addition of vegetable oils leads to a lower SFC at the beginning of the measurement compared to pure cocoa butter, which indicates that a smaller crystalline fraction is formed. This can be observed independent of the type of oil. The crystallizing component in the mixture is cocoa butter. Thus, a dilution with oils, which are all liquid at measuring temperature, leads to a reduced amount of TAG that can crystallize including those which start crystallization. In fast, dynamic, quasi-isothermal NMR measurements, the start of polymorphic transition can be recognized by the kink at which the slope of SFC over time increases. The kink appears at a lower SFC, but at the same measuring time, in samples with oil addition. This might be explained by the higher mobility, since the liquid TAG from the oil can act as a flow agent so that crystals are more mobile and the polymorphic transition starts earlier.
The formation and growth of instable crystals seems to be independent of the type of oil and is only affected by dilution. Subsequent polymorphic transition and maximum crystallization rate are decelerated by oils high in linoleic acid content such as walnut oil, followed by almond oil and are least affected by hazelnut and olive oil.
Post-crystallization Samples, investigated via fast, dynamic, quasi-isothermal NMR crystallization measurements proceed towards the same final value after 100 min measuring time, which confirms the results of SFC measurements with standardized sample preparation at 20 °C. Thus, the effects of different vegetable oils on crystal growth and polymorphic transition vanished during storage. Crystallization with standardized sample preparation (see chap. 3.6.2.2) can be seen as a reference measurement. The SFC with standardized sample preparation is not affected by different crystal growth rate or polymorphic transitions because it only refers to the sample composition. During the sample preparation instable crystals are melted and no further polymorphic transition from instable to stable forms occurs. Therefore, it can be concluded that the addition of vegetable oils causes a dilution that is directly proportional to the oil content.
The SFC measurements with standardized sample preparation of cocoa butter mixed with edible fats indicates the formation of a grain microstructure. Different crystals, starting from each component are formed and grow in the further course of cooling until they finally abut against each other. The SFC decreases much more than would be expected by sole dilution when increasing amounts of fat are added. Thus, the occurrence of an eutectic is likely. It can be identified at around 0.50 g/g for butterfat and 0.65 g/g for coconut fat addition. However, such high quantities are not relevant for production.
Summary Adding fats or oils, especially those with a low SFC, influences the crystallization process in many ways. The main crystallizing TAG in cocoa butter are of the sat-O-sat type. TAG with the same structure and chain length will be incorporated into the crystal lattice completely and even TAG with similar structure such as triolein (OOO) can be incorporated in small amounts. If TAG with different structure

and chain length are added, they seem to reduce the probability that matching TAG collide and form a crystal.
Besides the different melting ranges for edible fats and vegetable oils, a difference in FA and resulting TAG composition can be found. While the vegetable oils contained more unsaturated FA, their chain length is comparable to those found in cocoa butter. In contrast butterfat and coconut fat contain high amounts of short and medium chained FA, such as myristic or lauric acid. From this follows that vegetable oils mainly cause a dilution effect independent from the type of oil, but also have an impact on crystal growth depending on the saturation degree, which differs between the oils. However, the chain length of FA also affects crystal growth and seems to have an even stronger effect than the degree of unsaturation.
Initial fats and oils, which can get into the chocolate during production alter its crystallization. This changed crystallization might have an impact on the migration and subsequent fat bloom formation, which will be addressed in the following chapters.

4.2 Filling fat and oil detection in dark chocolate

The results in this section have been partly published in I. Rothkopf, J. Kind, et al. (2017). "Impact of sample preparation on physical quantification of filling fats and oils in fresh and stored chocolate". *European Journal of Lipid Science and Technology* 117.8. IGF-Vorhaben (17548 N), p. 1600359. ISSN: 1438-7697. DOI: 10.1002/ejlt.201600359.
For the quantification of the amount of migrated oil in chocolate, fast and efficient methods were needed to handle the sample amount. Thus physical and chemical methods were investigated and compared to find a suitable method.

4.2.1 Use of solid fat content (SFC) for filling fat and oil detection

The solid fat content (SFC) reduction caused by filling fats and oils can be used to quantify their amount in chocolate. The SFC of dark chocolate mixed with hazelnut oil, coconut fat and butterfat measured directly after β-stabilization procedure and after further storage for 22 days at 20 °C as described in chap. 3.6.2.2 is shown in fig. 4.8.

For all added fats and oils, the SFC decreases almost linearly with the amount of added fat. The slope differs, when samples are measured directly after preparation: hazelnut oil causes the least, butterfat the strongest decrease. The SFC of the same samples after further storage at 20 °C for 22 days has increased in total as can be seen in fig. 4.8. Additionally, the different effects on the SFC of the three filling fats and oils vanished. Therefore the decision and determination limit was also calculated combined for all three fats and oils. Since the slope is decreasing, decision and determination limit, which are described in chap. 3.3.2, are given as absolute values in tab. 4.5.
The SFC decrease with increasing amounts of added oil differs for cocoa butter or chocolate as base of the mixture. SFC reduction of 1 %(w/w) equals 10 mg/g oil addition in cocoa butter (see fig. 4.6), but only 5 mg/g oil addition in dark chocolate, which contained about 34 %(w/w) cocoa butter (see fig. 4.8).

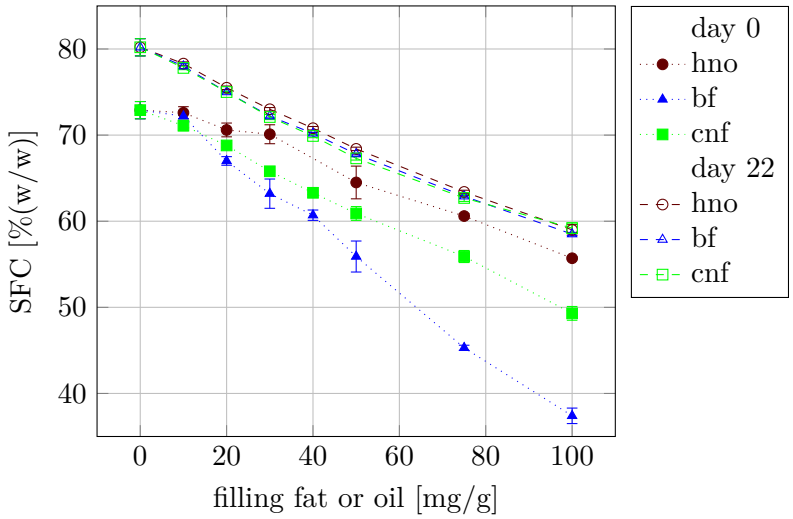


Figure 4.8: SFC of chocolate mixed with different amounts of hazelnut oil (hno), butterfat (bf) or coconut fat (cnf) related to whole sample amount, prepared with β-stabilization and measured immediately after preparation (day 0) and after 22 days storage at 20 °C

Table 4.5: Decision and determination limit with $\alpha = 0.05$ of hazelnut oil, coconut fat and butterfat in chocolate in mg/g quantified by SFC via NMR

		decision limit	determination limit
day 0	hazelnut oil	7	29±10
	coconut fat	3	12±4
	butterfat	4	14±5
	combined	11	49±16
day 22	hazelnut oil	3	12±4
	coconut fat	5	21±7
	butterfat	4	14±5
	combined	2	9±3

The SFC measurement was developed to investigate fats and oils. Thus, the other components in chocolate, such as sugar and fat free cocoa solids are not measured but the amount of filling fat or oil is based on the whole sample weight. Therefore, the differences of the amount of oil needed to lower the SFC by a certain value can be explained by the different fat content of the samples.

4.2.2 Fatty acid composition for quantification of filling oils and fats in chocolate

A typical method to investigate cocoa butter purity is chemical fatty acid (FA) or triacylglyceride (TAG) analysis. Cocoa butter with added fats and oils was analyzed. Fat and oil detection by FA analysis via gas chromatography (GC) is always based on the total fat amount, because the fat phase is dissolved in n-hexane while non-fat particles remain undissolved during sample preparation.
Using the ratio of two lead substance peak areas as suggested by Ziegleder, Moser, et al. 1996a eliminated shifts in the system. The lead substances should be chosen as follows: The first substance should be present in high amounts in the cocoa butter and low amounts in the filling fat or oil; the second should be vice versa. Stearic acid C18:0 was chosen as lead substance in cocoa butter and chocolate. For hazelnut oil the lead substance was oleic acid C18:1, for coconut fat it was lauric acid C12:0 and for butterfat butyric acid C4:0 as well as myristic acid C14:0 were used.
The ratio of the curve area of the chromatogram measured via GC of two FA, namely stearic C18 and oleic acid C18:1, was plotted against the mass fraction of hazelnut oil in chocolate that was set in the sample. The results are shown in fig. 4.9. Three samples were prepared per concentration and each measured in duplicate.

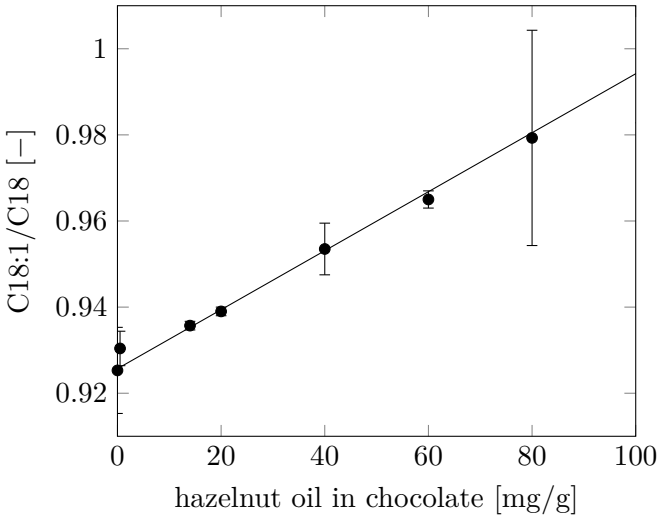


Figure 4.9: GC-FID calibration curve of hazelnut oil in dark chocolate as the ratio of the lead substances C18:0 for chocolate and C18:1 for hazelnut oil

The decision and determination limit was calculated as described in chap. 3.3.2 for each filling fat or oil as well as the correlation coefficient R^2, listed in tab. 4.6. These values were used to identify the most efficient method and to assure the reliability of the measured differences by these methods in chap. 4.3.

Table 4.6: Decision and determination limit with $\alpha = 0.05$ of hazelnut oil, coconut fat and butterfat (lead substance in brackets) in dark chocolate (lead substance C18) in mg/g quantified by FA composition via GC with number of concentrations c and samples s for each concentration and correlation coefficient R^2 calculated for the ratio of the lead substances to given concentration

	decision limit	determination limit	c	s	R^2
hazelnut oil (C18:1)	0.26	0.91 ± 0.30	6	7	0.9976
coconut fat (C12)	0.03	0.11 ± 0.04	6	7	0.9996
butterfat (C4)	1.333	3.67 ± 1.22	3	4	0.9818
butterfat (C14)	0.19	0.67 ± 0.22	6	6	0.9927

Butyric acid C4 is a lead substance for butterfat. However, it could only be detected in amounts higher than 4 mg/g sample. Therefore C14 was also used as a lead substance. To ensure applicability, mixtures of CB 01 and CB 03 with hazelnut oil were analyzed and results are shown in fig. 4.10. The regression is linear for both samples, but absolute values differ. This is caused by the varying concentration of the lead substance C18 in the cocoa butter samples. Thus, a new calibration is needed for every cocoa butter.

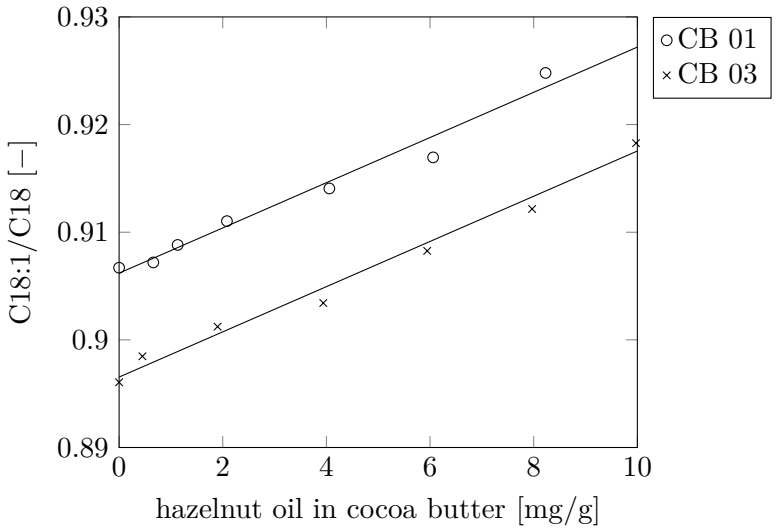


Figure 4.10: GC calibration curve of hazelnut oil in cocoa butter CB 01 and CB 03

The recovery rate (RR) from several measurements was calculated using eq. 3.3.1. The

amount of filling fats and oils was calculated using the calibration curves and compared to the given amount. Results are shown in fig. 4.11. The expected RR is 1 g/g. The RR was stable for amounts of 2 mg/g to 10 mg/g oil or fat in chocolate.

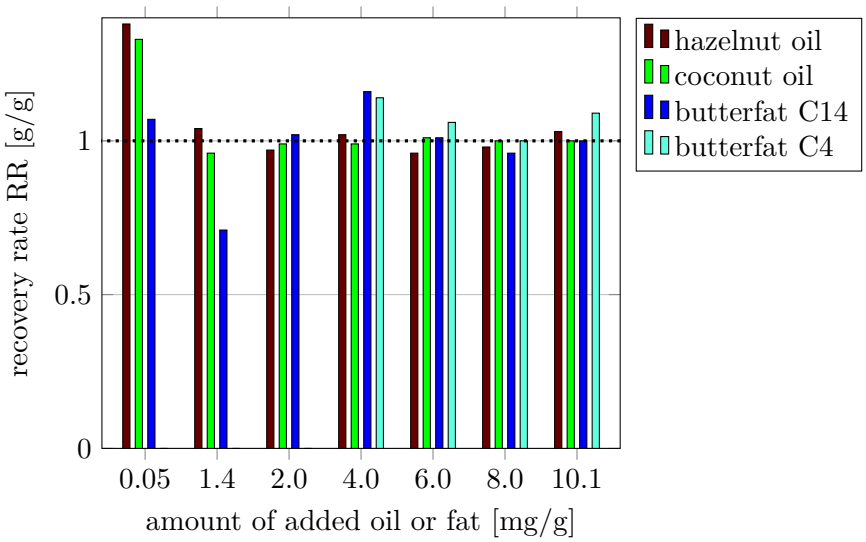


Figure 4.11: Recovery rate for GC-FID measurements with target recovery rate of 1 g/g

4.2.3 Discussion

Solid fat content (SFC) Fillings for chocolate products are often soft and have a lower SFC than the firm chocolate to achieve an interesting texture. The SFC of a mixture of two substances with different SFC lies in between that of the pure substances, if no eutectic effects occur (Gordon et al. 1979; Hogenbirk 1984). Rothkopf and Danzl 2015 showed, that the SFC reducing effect is the same up to an amount of 0.5 g/g of added filling fat or oil. However, this could not be observed when samples were measured directly after sample preparation, but after several days of storage due to equilibration. During the fast crystallization of the chocolate, a fraction of TAG from the filling fats and oils might be integrated in the crystal lattice of the fat in chocolate (Rothkopf and Danzl 2015; Ziegleder, Geier-Greguska, et al. 1994). Since the investigated fats and oils show different compatibilities, their SFC reducing effect varies (Rothkopf and Danzl 2015). Regarding the progression over time post-crystallization can be observed (Strassbourg et al. 2006). Filling fats and oils seem to have an impact on this process.
The varying degrees of SFC reduction caused by different types of filling fats and oils level out during storage. This can also be seen regarding the decision and determination limit across all investigated fats and oils. This leads to the assumption that the filling fats and oils are disintegrated from the crystal lattice over time independently of their type, while TAG of the sat-O-sat-type are integrated. The SFC reduction caused by oil addition in cocoa butter compared to chocolate shown in chap. 4.1 in fig. 4.6, suggests

that the added oil only acts as a dilution agent. Therefore, this method can be used to detect and quantify vegetable oils in chocolate or cocoa butter.

Fatty acid composition determined by GC The reliability of chemical methods, such as GC measurements for FA detection was investigated by measuring calibration curves with given amounts of filling fats and oils. Therefore decision and determination limit were calculated using the ratio of lead substances to evaluate detectable and quantifiable amounts of filling fats and oils. The usage of the peak ratio of the lead substances showed linear, precise and reliable results for the GC method. Additionally, systemic fluctuations are reduced by regarding a peak ratio rather than by using absolute signals. However, the calculated detection and determination limits are only valid for the fats and oils used in this study. Fluctuation in fat composition affect detection and determination limits. A higher concentration of the lead substances will reduce both limits. On the other hand it was shown that the method is also valid for other mixtures, e.g. other cocoa butter samples. However, a calibration curve with defined mixtures must be recorded in advance and after each change in the apparatus.

Comparison of physical and chemical methods The detection of hazelnut oil in chocolate and cocoa butter is more accurate than that of butterfat and coconut fat. Therefore hazelnut oil will be used for the further experiments. It was also shown that chemical methods for detection were much more accurate and can already detect one tenth of the amount compared to physical methods. However, the effort for the chemical methods is much higher than for the physical detection methods. For this reason, physical methods will be used primarily for large sample quantities, while chemical methods will be used for high accuracy requirements.

4.3 Filling oil migration in chocolate

The migration mechanism of oil in chocolate is still not fully understood. Therefore, migration was measured in cylindrical model systems described in chap. 3.4.1. Both the continuous and the dispersed phase of the cylinders were varied by changing their composition. Chocolate and pure cocoa butter CB 04 as well as a mixture of both was investigated as the *basic experiment* to study the impact of particle amount. The variation of the amount of particles allows to distinguish between capillary flow along the particle surface and diffusion through the fat phase. An increase of migrated oil with increasing particle amount indicates capillary flow along the particle surface as the predominant mechanism, while decreased migration with increasing particle amount would indicate diffusion through the fat phase. The predominant polymorphic form in these samples was β_{V} in the *basic experiment* and β_{VI} in another sample set since the morphology of crystals in these polymorphic forms may affect migration. To further study the impact of the type of particle and their surface properties, cocoa butter was mixed with fat-free cocoa powder (D-00-ZR, ADM International Sarl, Rolle, Swiss) or finest sugar (SF0, Pfeifer & Langen, Cologne, Germany) and the particle surface properties were additionally changed by adding lecithin. The samples are listed in tab. 3.2.

The migration rate depends on temperature, thus samples were stored at 18 °C, 20 °C

and 23 °C and three slices of each 5 mm were investigated on migrated hazelnut oil amount by gas chromatography (GC) and nuclear magnetic resonance (NMR) analysis. In general, it was observed that in some cases the nougat adhered to the bottom of the cylinders, which can be seen in fig. 4.12. Thus, the contact area and intensity might be different for each cylinder.

Figure 4.12: Picture of two cocoa butter, two chocolate and two 1:1-mixture cylinders (from left to right) with adherent nougat at the bottom (Sonnleitner 2016)

In cylinders made of cocoa butter with finest sugar and fat free cocoa powder, some white spots could be observed when cut in half, as shown in fig. 4.13.

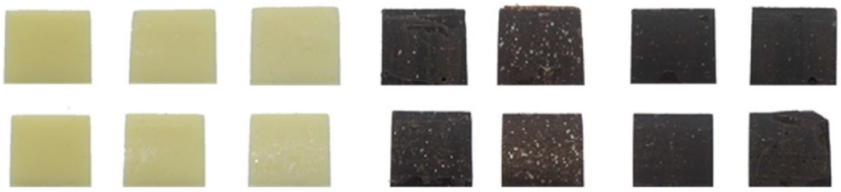


Figure 4.13: Pictures of the sectional view of cylinders made of cocoa butter and mixtures with sugar (S) and cocoa powder (CP), S30_CP0, S50_CP0, S10_CP20, S25_CP25, S0_CP30 and S0_CP50 (from left to right) (Waldschütz 2016)

4.3.1 Storage temperature, time and sampling

At first the impact of the storage temperature on the migration rate in the slices was investigated in cocoa butter CB 04 and pure chocolate. The amount of migrated hazelnut oil indicated by solid fat content (SFC) obtained via NMR is shown in fig. 4.14 for the bottom slice of cocoa butter stored at 18 °C, 20 °C and 23 °C.
The SFC, determined by NMR, increases during the first week of storage and decreases during further storage. Higher storage temperatures cause a stronger decrease of the SFC. The storage temperature affects the migration rate.

The SFC obtained via NMR (after 21 d storage) of the top, middle and bottom slices of a cocoa butter cylinder is shown in fig. 4.15. It can be seen, that the most changes occur in the bottom slice, while the middle and top slice are rarely affected.

4.3.2 Basic experiment - chocolate and cocoa butter

To identify the migration mechanism the particle amount in the samples was varied by comparing cocoa butter without particles to chocolate with about 0.66 g/g particles and

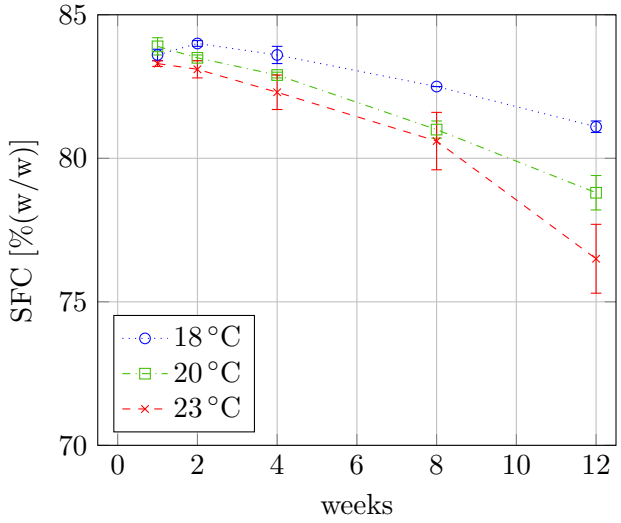


Figure 4.14: SFC of the bottom slice of cocoa butter model cylinders stored at 18 °C, 20 °C and 23 °C, determined by NMR analysis after sample preparation with β-stabilization and 21 d storage at 20 °C determined in duplicate

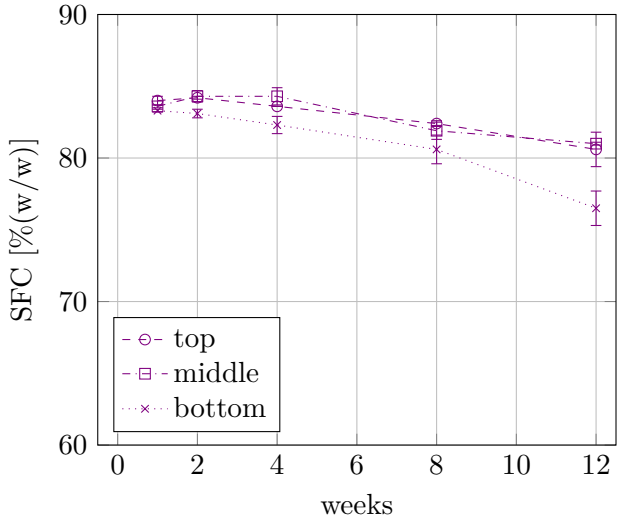
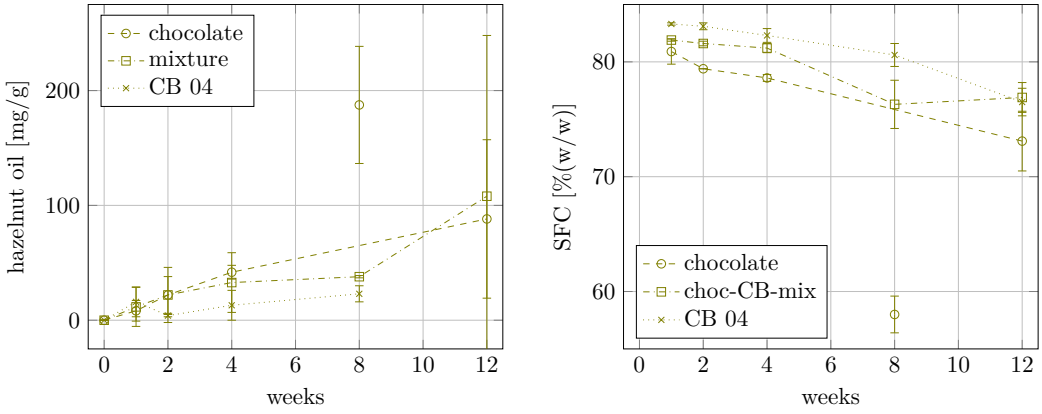


Figure 4.15: SFC of the bottom, middle and top slices of cocoa butter model cylinders stored at 23 °C, determined by NMR after sample preparation with β-stabilization and 21 d storage at 20 °C determined in duplicate

a 1:1 mixture of both with about 0.33 g/g particles. Results are shown in fig. 4.16a and fig. 4.16b for GC and NMR analysis, respectively.



(a) hazelnut oil content via GC

(b) SFC via NMR

Figure 4.16: SFC and amount of hazelnut oil that migrated into the bottom slice of basic experiment dark chocolate, cocoa butter and 1:1 mixture model cylinders stored at 23 °C, determined by GC analysis in triplicate and by NMR analysis after sample preparation with β-stabilization and 21 d storage at 20 °C determined in duplicate

The comparison of chocolate, cocoa butter and the 1:1 mixture of both shows an increasing hazelnut oil content with increasing particle amount determined by GC. The change of SFC during storage is almost the same for all samples. However, the chocolate sample after 8 weeks storage shows an outlier for both methods. Comparing chocolate and cocoa butter, the hazelnut oil content based on the fat phase is much higher in chocolate than in cocoa butter. Along with this the SFC reduction in chocolate is bigger than in cocoa butter. Additionally, the SFC decrease is more pronounced with longer storage time.

4.3.3 Dispersed phase - variation of type and amount of particles

Sugar and cocoa particles show differences in hydrophobicity, porosity and surface roughness. To study the impact of particles, model systems with fat-free cocoa powder (D-00-ZR, ADM International Sarl, Rolle, Swiss) and finest sugar (SF0, Pfeifer & Langen, Cologne, Germany) in cocoa butter were prepared. The particle size, Brunauer Emmett Teller (BET)-surface and free liquid fat content were determined and migration trials were performed. The impact of lecithin was also investigated, because most chocolates are produced with lecithin.

4.3.3.1 Particle size distribution and BET-surface

The circle equivalent (CE) diameter and volume based particle density distribution of fat free cocoa powder and finest sugar measured as described in chap. 3.6.5, are shown in fig. 4.17.
The particle size distribution of the finest sugar is narrower than that of the cocoa powder, which means that the sugar contains fewer very small and very big particles. Both show a maximum just below 20 µm. The particles were measured in a wet cell with oil. Therefore, agglomeration of the particles might occur, which is more likely to happen for sugar due to the hydrophilic particle surface (Tscheuschner and Markov 1989). The optical measuring system allowed to visually look at the particles identified by the system and differ between agglomerates and particles. This makes it possible to exclude agglomerates for the particle size evaluation.

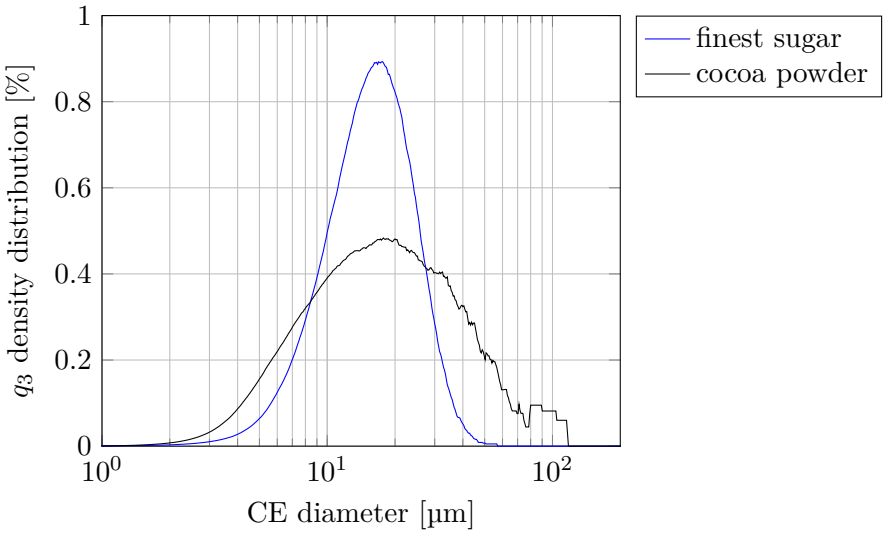


Figure 4.17: CE diameter and volume based particle density distribution q_3 of fat free cocoa powder and finest sugar

The externally measured BET-surface (see chap. 3.6.6) of fat free cocoa powder (D-00-ZR, ADM International Sarl, Rolle, Swiss) is 3.805 m^2/g and 0.722 m^2/g for finest sugar (SF0, Pfeifer & Langen, Cologne, Germany). The specific surface of cocoa particles is more than 5 times bigger than that of the sugar particles. Thus, the resulting surface area of the particles present in the mixtures can be calculated and is shown in tab. 4.7. The comparable particle size distribution and the huge differences in BET-surface confirm that sugar has a smooth surface while the cocoa particles have a rougher surface with pores, which was also found by Do et al. 2011.

Table 4.7: Increasing BET-surface of particles in cocoa butter mixed with different amounts of finest sugar (S) and fat-free cocoa powder (CP)

model system	sugar [g/g]	cocoa powder [g/g]	BET-surface [m^2/g]
S30_CP0	0.30	0.0	0.2166
S50_CP0	0.50	0.0	0.361
S10_CP20	0.10	0.20	0.8332
S25_CP25	0.25	0.25	1.1318
S0_CP30	0	0.30	1.1415
S0_CP50	0	0.50	1.9025

4.3.3.2 Free fat

The amount of free fat was determined as described in chap. 3.6.4 with a centrifuge and is shown in fig. 4.18. Samples are sorted according to their particles BET-surface area.

The particle type, the specific particles surface, the size of the particles and the amount of lecithin is of importance for the free fat content (Tscheuschner and Markov 1989). For the model systems with 0.5 g/g particles, namely S50_CP0, S25_CP25, S0_CP50, a decrease in free fat with increasing BET-surface area of the particles in the sample can be observed. This is also the case for model systems with 0.3 g/g particles, namely S30_CP0, S10_CP20, S0_CP30. However, the model systems with 0.3 g/g particles show an overall higher amount of free fat compared to the model systems with 0.5 g/g particles. The impact of lecithin was only significant for S50_CP0 and S0_CP30.

The particle size distribution and BET-surface area are supposed to have an impact on the free fat amount. However, the mixtures S25_CP25 and S0_CP30 show almost the same BET-surface area and have comparable particle size distributions, but show different free fat amounts. Thus, the particle amount, particle-particle and particle-liquid interaction and surface charge seem to affect the free fat amount determined by centrifuging. Comparing the samples with 0.5 g/g particles, a decrease of free fat with increasing BET-surface can be observed. The samples with higher amounts of cocoa powder have a lower fraction of free fat, which can be explained by the higher surface area. However, the increase in free fat with increasing cocoa powder amount cannot be fully explained by the increase in BET-surface. The BET-surface area in sample S0_CP50 is five times larger than that of S50_CP0, while the free fat amount is only two and a half times lower. This may be explained by pores in which the fat is trapped (Do et al. 2011). Additionally the fat might be stronger bound to the lipophilic cocoa powder surface than to the hydrophilic sugar surface. The same can be observed for samples with 0.3 g/g particles, but to a lesser extent. The free fat content of around 0.6 g/g to 0.8 g/g is high and the difference in cocoa particle amount is small.

The difference of samples with 0.3 g/g and 0.5 g/g particles might be caused by particle interaction. Beside the fat binding properties of cocoa powder, agglomeration of

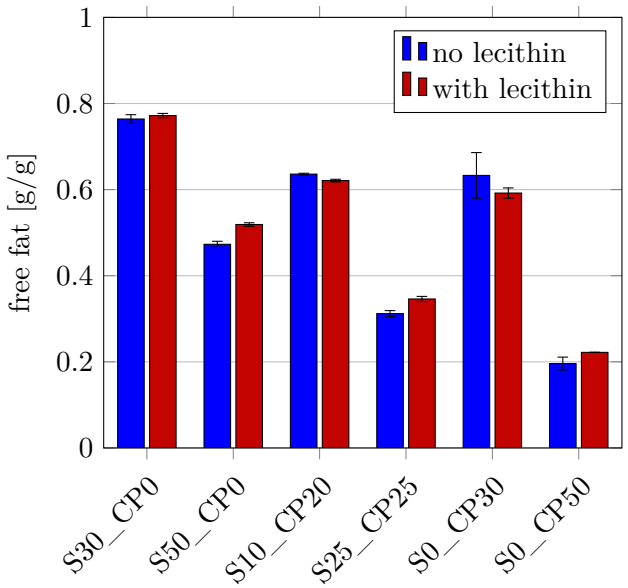


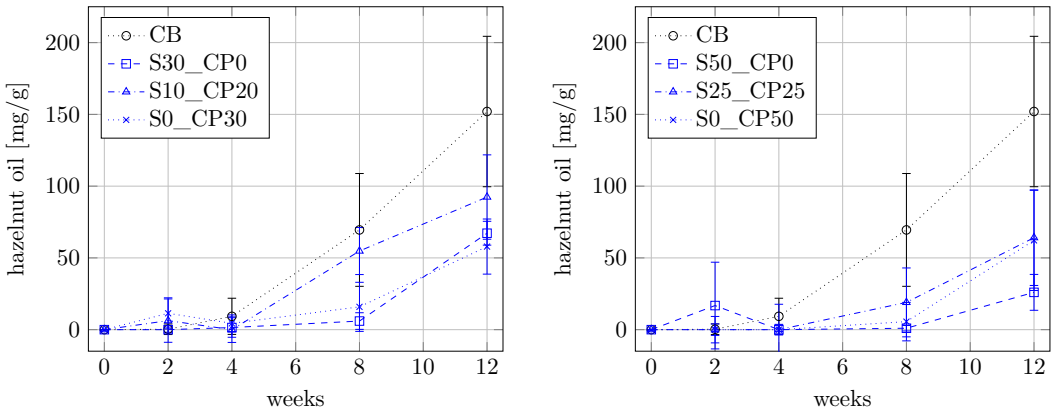
Figure 4.18: Free fat based on fat content of cocoa butter, sugar and cocoa powder mixtures, according to tab. 3.2 with 5 mg/g or without lecithin determined by centrifuge, sorted according to BET-surface

the hydrophilic sugar particles in the lipophilic continuous cocoa butter might occur. In these agglomerates cocoa butter can be enclosed (Tscheuschner and Markov 1989; Tscheuschner 1993).
The effect of the addition of lecithin on free fat content was investigated to ensure the comparability of the model systems and chocolate, which often contains lecithin. However, lecithin addition only had a small impact on the free fat content.

4.3.3.3 Migration trials with varied type and amount of particles

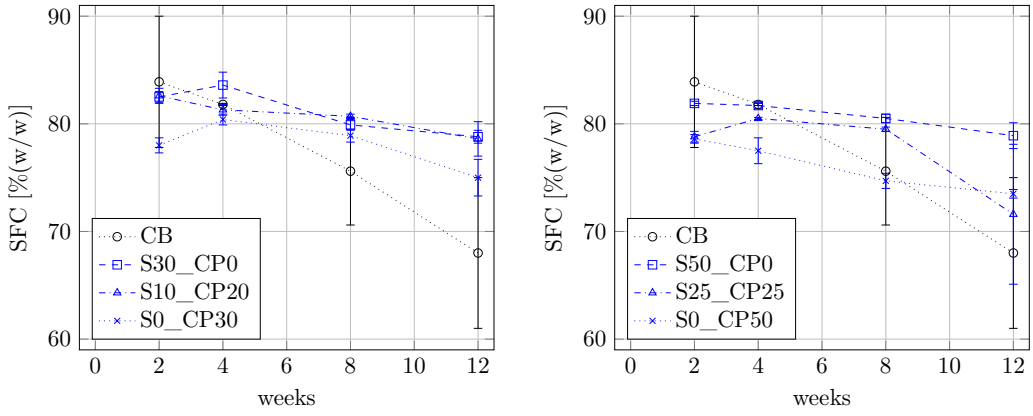
The results of the migration trials are shown in fig. 4.19 and fig. 4.20 for GC and NMR analysis, respectively. The values of the cylinder model of pure CB 04 , already shown in fig. 4.14 for storage at 23 °C are included as a reference.

The GC analysis shows that the most hazelnut oil migrated in pure cocoa butter. The least amounts of hazelnut oil were found in model systems with only sugar or only cocoa powder. The hazelnut oil migration in model systems with a mixture of sugar and cocoa powder particles is in between pure cocoa butter and the model system with one type of particle. In model system cylinders with 0.5 g/g particles less hazelnut oil was detected than in model systems with 0.3 g/g particles. Migration during storage is also affected by the amount of particles.
NMR analysis also showed that the SFC of pure cocoa butter was reduced the most compared to the other samples. For the particle containing model systems no clear



(a) 0.3 g/g particles (b) 0.5 g/g particles

Figure 4.19: Amount of hazelnut oil in mg/g based on fat content that migrated into the bottom slice of the cocoa butter CB 04 model cylinders with different amounts of sugar and fat-free cocoa powder particles, according to tab. 3.2, stored at 23 °C, determined by GC analysis in triplicate



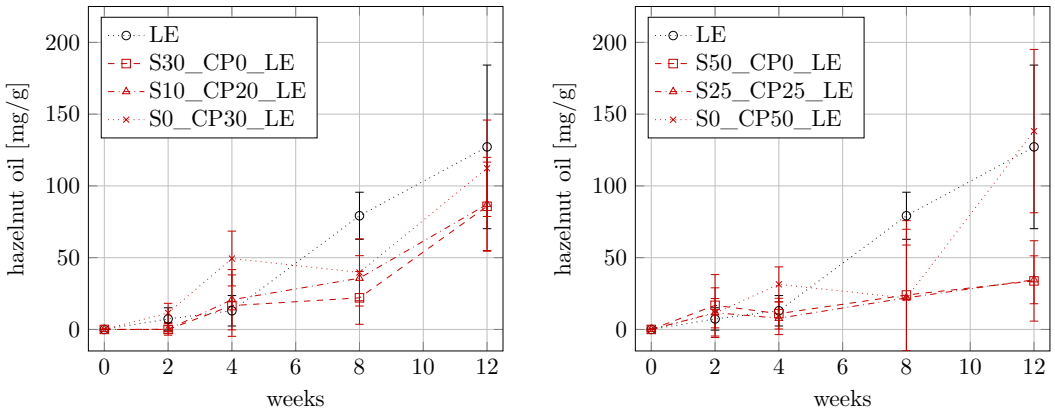
(a) 0.3 g/g particles (b) 0.5 g/g particles

Figure 4.20: SFC of the bottom slice of cocoa butter CB 04 model cylinders with different amounts of sugar and fat-free cocoa powder particles, according to tab. 3.2, stored at 23 °C, determined by NMR analysis after sample preparation with β-stabilization and 21 d storage at 20 °C determined in duplicate

trend could be observed due to high standard deviation. The SFC of samples with 0.3 g/g particles was reduced to a lower amount than the SFC of model systems with 0.5 g/g particles.

4.3.4 Changed particle surface properties by adding lecithin

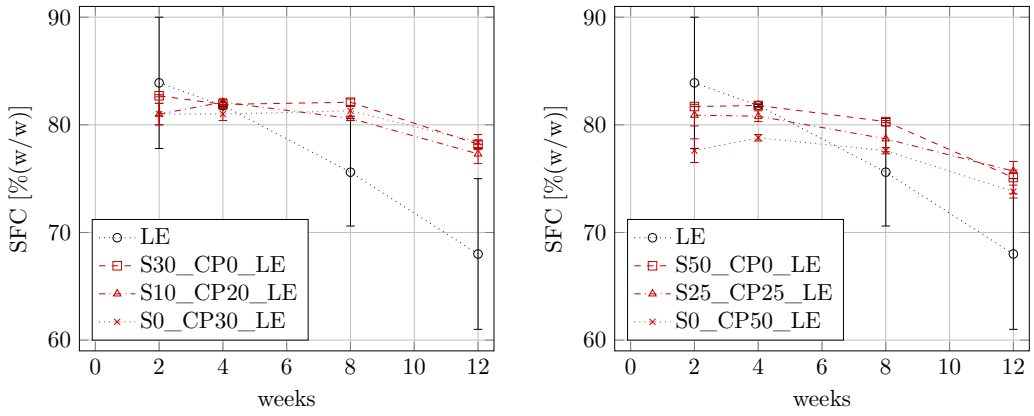
Particle surface properties are affected by the type of particles, but can also be modified by adding lecithin, which is usually done in chocolate. Lecithin is known to accumulate on the surface of sugar particles and interact with the surrounding cocoa butter. Thus, the proportion of bound fat in a cocoa butter-sugar dispersion can be increased by lecithin (Middendorf et al. 2016). Lecithin might also bind migrating oil to the sugar surface. For this reason it was added to the model cylinders and migration was investigated. Results are shown in fig. 4.21 and fig. 4.22 for GC and NMR measurements, respectively.



(a) 0.3 g/g particles (b) 0.5 g/g particles

Figure 4.21: Amount of hazelnut oil that migrated into the bottom slice of cocoa butter CB 04 model cylinders with different amounts of sugar and fat-free cocoa powder and with 5 mg/g lecithin stored at 23 °C, determined by GC analysis in triplicate

Compared to model cylinders without lecithin migration detected via GC is slightly decreased for model cylinders with sugar or with a mixture of sugar and cocoa powder. Model cylinders with cocoa powder and lecithin had higher amounts of hazelnut oil compared to cylinders without lecithin. However, the differences are not significant.
SFC values determined via NMR showed less standard deviation for model cylinders with lecithin compared to cylinders without lecithin. For systems with 0.3 g/g particles there is no clear trend with regard to the type of particles. In comparison, model cylinder systems with 0.5 g/g particles showed stronger SFC reduction. In these model cylinder systems the SFC of cocoa butter with cocoa powder S0_CP50_LE is least reduced, followed by S25_CP25_LE and S50_CP0_LE, which show comparable SFC reduction



(a) 0.3 g/g particles (b) 0.5 g/g particles

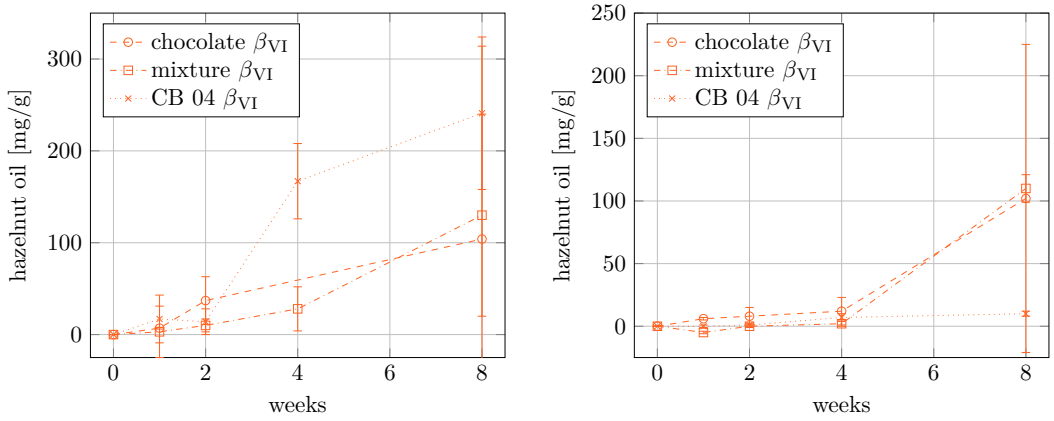
Figure 4.22: SFC of the bottom slice of cocoa butter model cylinders with different amounts of sugar and fat-free cocoa powder with 5 mg/g lecithin stored at 23 °C, determined by NMR analysis after sample preparation with β-stabilization and 21 d storage at 20 °C in duplicate

and pure cocoa butter showed strongest SFC reduction during storage time.

4.3.5 Impact of cocoa butter polymorphism

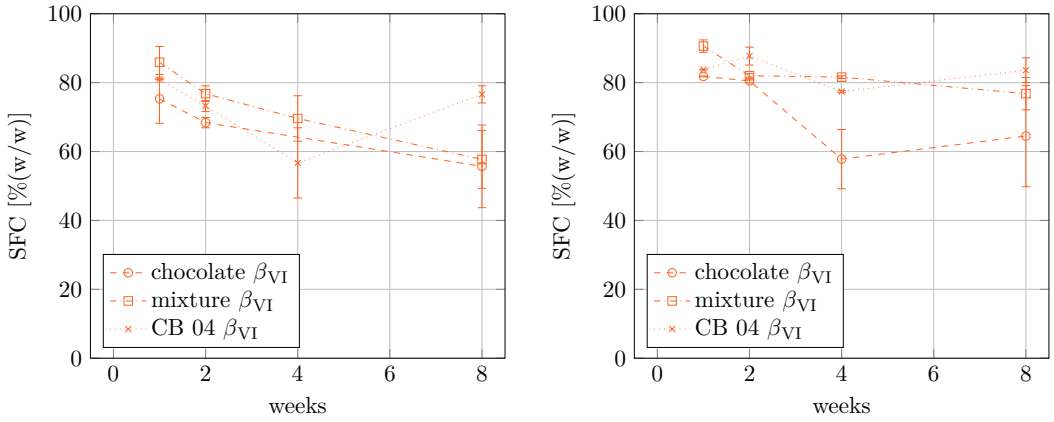
K. W. Smith, Cain, et al. 2007 reported, that migrating hazelnut oil accelerates the polymorphic transition of cocoa butter. It is yet unclear how the polymorphic transition affects migration. Therefore the basic experimental set up was repeated with cylinders, that were stored at 27 °C to force polymorphic transition. The transition from β_V to β_{VI} was investigated by differential scanning calorimetry (DSC) melting curves. While it took about 76 d for dark chocolate to transform into β_{VI} polymorph, the polymorphic state in cocoa butter was changed after 21 d and the 1:1 mixture was completely transformed in the β_{VI} polymorph after 28 d. After these time periods the cylinders were placed on the nougat base and stored at 18 °C, 20 °C and 23 °C. Results are shown in fig. 4.23 for GC analysis and in fig. 4.24 for NMR measurements.

During the storage of the cylinders on the nougat a softening and swelling of the cylinders was observed. This resulted in sampling difficulties and high standard deviation. Therefore the results of the middle slice are also shown. It can be seen that samples with increasing particle amount show increased values of hazelnut oil determined by GC. Moreover, the amount of migrated hazelnut oil is much higher in samples in β_{VI} state than for those in β_V state (shown in fig. 4.16). The SFC determined by NMR shows a similar trend. With increasing particle amount the SFC is more reduced. The SFC reduction caused by added hazelnut oil is twice as high for chocolate as for cocoa butter (shown in chap. 4.2).



(a) bottom slice (b) middle slice

Figure 4.23: Amount of hazelnut oil that migrated into the bottom and middle slices of dark chocolate, cocoa butter and 1:1 mixture model cylinders in β_{VI} polymorphic form stored at 23 °C, determined by GC analysis in triplicate



(a) bottom slice (b) middle slice

Figure 4.24: SFC of the bottom and middle slices of dark chocolate, cocoa butter and 1:1 mixture model cylinders in β_{VI} polymorphic form stored at 23 °C, determined by NMR analysis after sample preparation with β-stabilization and 21 d storage at 20 °C determined in duplicate

4.3.6 Discussion

Storage conditions For all systems it was confirmed that the amount of migrated hazelnut oil increased with higher storage temperatures and longer storage times, which was also described by other authors (Ali et al. 2001; Choi, K. L. McCarthy, and M. J. McCarthy 2005; Dahlenborg, Millqvist-Fureby, et al. 2015b; Khan and Rousseau 2006; Miquel et al. 2001). A higher amount of the fat phase is liquid at higher temperatures and diffusion of the oil from the nougat base into the liquid fatty phase of the cylinder might occur. The viscosity is also decreased at higher temperatures, which increases the diffusion rate as well as capillary flow and results in a higher migration rate (Ziegleder, Moser, et al. 1996b). The migrated amount of oil is lower with increasing distance to the nougat base. The thickness of each slice was 5 mm, which shows that the hazelnut oil migrates only a few mm during the storage time of 12 weeks.
The migration in dependency of distance was also investigated within distances of 0 mm to 2.5 mm (Adenier et al. 1993; Dahlenborg, Millqvist-Fureby, et al. 2015b; K. W. Smith, Cain, et al. 2007; Marty, Baker, et al. 2005; Svanberg et al. 2011a). However, Marty, Baker, et al. 2005 also found an exponential decrease in the amount of migrated fat for up to 7 mm from the filling oil base.
Basic experiment - chocolate and cocoa butter The migration mechanism in chocolate is still not fully understood. Thus, a model system with different amounts of particles was used. Diffusion through the fat phase is faster in pure cocoa butter due to less tortuosity. Migration caused by capillary flow through pores or along the particle surface is accelerated by the presence of particles in the sample, as was expected.
The particle amount in the model systems of the basic experiment can be estimated by the fat content. The dark chocolate contains around 0.34 g/g fat and results in 0.66 g/g particles. Thus, the 1:1 mixture of chocolate and cocoa butter contains 0.33 g/g particles. The amount of migrated hazelnut oil was slightly higher in the dark chocolate samples than in the mixture and in the pure cocoa butter. Thus migration along the particles seems to be the transport mechanism (Aguilera et al. 2004). However, the higher amount of migrated hazelnut oil is not directly proportional to the amount of particles in the sample. This leads to the assumption that a minimum particle amount is needed, so that the particles can interact and form a network. Additionally, the particles themselves can be regarded as a porous network, which enables hazelnut oil migration (Aguilera et al. 2004). However, it has to be considered that the SFC reduction detected by NMR, which was caused by hazelnut oil, is half for cocoa butter compared to chocolate, as was described in chap. 4.2.1.
Dispersed phase - variation of type and amount of particles Chocolate contains sugar as well as cocoa particles and the impact on migration might be different. Sugar crystals have a smooth surface and dense structure without pores, while cocoa particles are highly porous and have a rough surface (Do et al. 2011). Thus model systems with a mixture of cocoa butter with fat-free cocoa powder or finest sugar were used to study the impact of particle type. The total and individual amount of particles was also varied and pure cocoa butter was used as a reference.
In contrast to the experiments with chocolate and cocoa butter mixtures, pure cocoa

butter showed the highest amount of migrated hazelnut oil, while samples with particles contained lower amounts of migrated hazelnut oil. This was also observed by Dahlenborg, Millqvist-Fureby, et al. 2015b. The results of the experiments with samples made of cocoa butter with added fat-free cocoa powder or finest sugar lead to the assumption that migration can be described by diffusion through the fat phase. Due to the particles, a higher tortuosity is expected, which extends the migration path (Galdámez et al. 2009; Ghosh et al. 2002; Motwani et al. 2011). The slices in this study might have been too thick to see this effect and the surplus slice leads to a dilution of the migrated hazelnut oil. A thick slice with a high amount of hazelnut oil in the lower part might result in the same amount of migrated hazelnut oil as a slice with the same thickness and an equally distributed low amount of hazelnut oil in the whole slice.
The increased hazelnut oil migration rate is not proportional to the larger particle surface area. This was suggested due to the higher oil binding capacity of an enlarged surface and the differences in hydrophobic surface properties of sugar and cocoa powder. The particle size distribution might also be important, because smaller particles can fill up the space between the bigger particles, which causes a denser packaging (Hartel 1999).
Changed particle surface properties by adding lecithin The migration rate for samples with lecithin addition were not significantly different due to the high standard deviation. The tendency of slightly increased migration rates for sample mixtures with sugar indicate an effect on samples with particles with lipophobic surface properties. Lecithin might help to break up agglomerates and increases the interaction of sugar and fat at the surface (Greiner et al. 2014; Timms 2002). However, the effect is very low.
Impact of cocoa butter polymorphism An interference of altered crystallization and changed migration caused by particle addition might be the reason for contradictory findings in literature and in this study compared to literature (Reinke, Hauf, et al. 2015; Rousseau and P. Smith 2008; Sonwai and Rousseau 2006; Deka et al. 2006). Another possibility is a different sample treatment, such as deareation by vibration or particle treatment. However, an altered crystallization and migration affect the microstructure of a system. Cocoa butter forms a porous network during cooling due to contraction (Lee et al. 2010). This is especially the case if a polymorphic transition occurs (Lovegren and Feuge 1965; Svanberg et al. 2011a).
The samples were stored at elevated temperatures during preparation to accelerate the polymorphic transition from β_{V} to β_{VI} . It took longer for the dark chocolate to transform into β_{VI} than it took for the 1:1 cocoa-butter-chocolate-mixture and the pure cocoa butter. This was also observed for β'_{III} to β_{V} transition by Ziegleder and Kegel 1989. Particles might not only influence the migration, but also the polymorphic transition.
The migration rate in all samples with β_{VI} was higher than in the samples with β_{V} crystals, independent from the particle amount. However, the polymorphic form might not be the sole reason but also the accompanying structural changes. Latent heat is released during the polymorphic transition and smaller crystals melt, which results in Ostwald ripening. Additionally β_{VI} has a denser structure than β_{V} and the crystal lattice contracts. This can cause cracks and crevices, which creates pores that allow capillary flow. These cracks were found by Reinke, Wilde, et al. 2015 using synchrotron x-ray micro-

tomography, as images with a light microscope are not possible due to the dark color. Reinke, Hauf, et al. 2015 assumed that migration occurs through these pores in the beginning followed by diffusion through the fat phase. Additionally β_{VI} shows needle like crystals while β_{V} has nearly spherical ones (Arishima and Sato 1989). The hazelnut oil might easily migrate along the needle shaped crystals without steric hindrance.
However, it remains unclear if the increased migration rate in samples with β_{VI} crystal form is caused by the polymorphic transition or by the accompanying structural changes. This might only be clarified if samples can be directly crystallized from the melt in form β_{VI} , which could not be done until today van Malssen, Langevelde, et al. 1999.
General conclusion Migration in chocolate is affected by the properties of all components. The crystal structure of cocoa butter depends on the chemical composition, the tempering process and further sample treatment such as cooling. Particles also have an impact on cocoa butter crystal structure and additionally affect migration due to their particle size, surface properties and porosity. Additionally, sample processing, such as grinding or conching, seems to have an impact and will be subject to ongoing research.

4.4 Filled chocolates

The interaction of migration and crystallization may occur during production and storage. In the following the effects of storage conditions, production parameters and initial hazelnut oil on migration, crystallization and fat bloom development during storage will be shown. For the experiments *pralines* (filled chocolate) as well as *shells* (only chocolate without filling) were produced and stored. For some measurements the filling had to be removed from the pralines and only the *emptied shells* are measured.

4.4.1 Storage tests

The shelf life of filled chocolates is mainly limited by physical changes such as softening of the chocolate shell or fat bloom formation. However, these changes do not occur immediately after production, but during storage. Thus, optimal storage conditions for accelerated storage tests had to be identified by storage tests with temperature variation. Dark chocolate pralines with nougat filling as well as pure shells without filling were stored isothermally and at cycling storage temperature. The solid fat content (SFC) was analyzed as an indicator for the amount of migrated hazelnut oil from the filling (see chap. 3.6.2) and the Whiteness Index (WI) was used as an indicator for fat bloom formation (as described in chap. 3.6.7).

4.4.1.1 Solid fat content (SFC) as indicator for migrated oil in stored pralines

The SFC can be used as an indicator for migrated hazelnut oil, as shown in chap. 4.2. The SFC of emptied chocolate shells, which were separated from the nougat after storage, is shown in fig. 4.25 for isothermally stored pralines and in fig. 4.26 for pralines, which were subject to cycling storage temperatures.

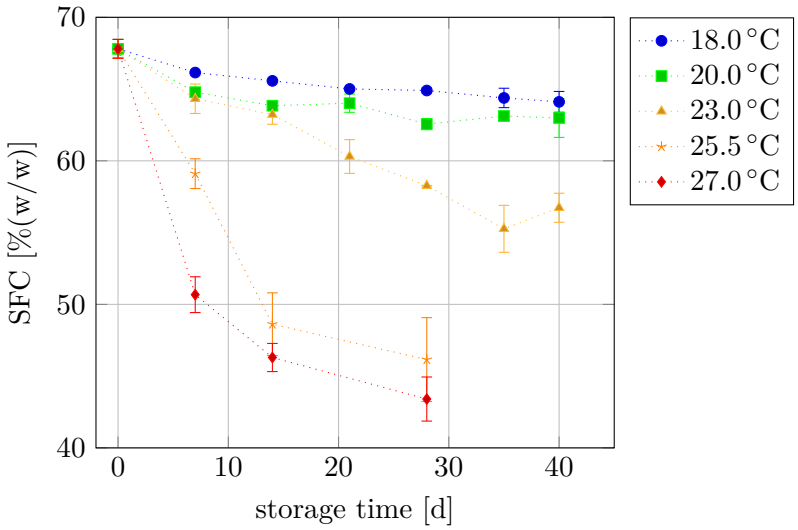


Figure 4.25: SFC over time of emptied chocolate shells obtained from isothermally stored pralines

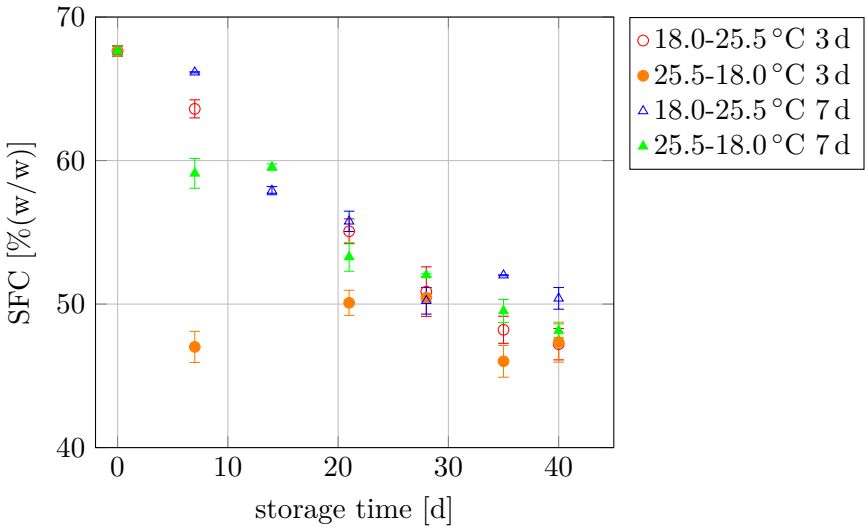


Figure 4.26: SFC over time of emptied chocolate shells obtained from pralines subjected to cycling temperature storage

Rising isothermal storage temperature leads to a faster decrease of SFC over time, as can be seen in fig. 4.25. In contrast, the SFC decrease is independent from the starting cycling temperature and cycling frequency after storage for 21 d, which is shown in fig. 4.26. The SFC of emptied chocolate shells from pralines stored at 25.5 °C for 3 d followed by storage at 18 °C for further 3 d was noticeably low. This SFC was even lower than the SFC of emptied shells from pralines, which were stored isothermally at 27 °C. The SFC of emptied chocolate shells obtained from pralines after 28 d storage can be seen in fig. 4.27. The SFC of emptied chocolate shells from pralines stored at cycling temperatures is in between that of emptied chocolate shells from pralines, which were stored at corresponding isothermal temperatures, namely 18.0 °C and 25.5 °C.

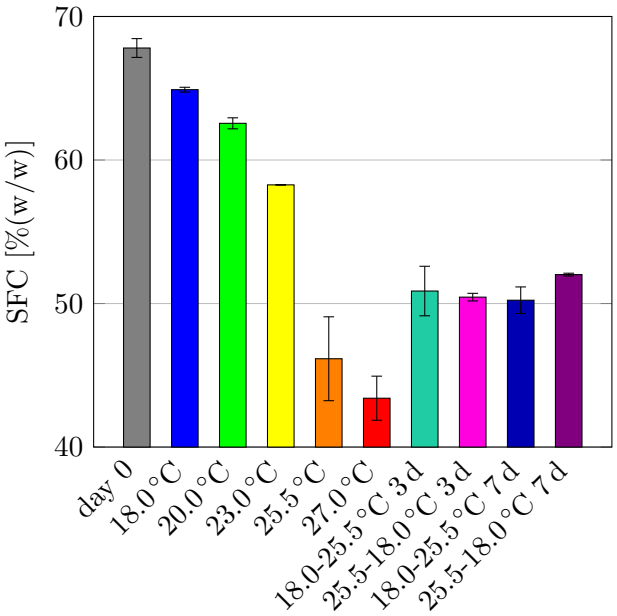
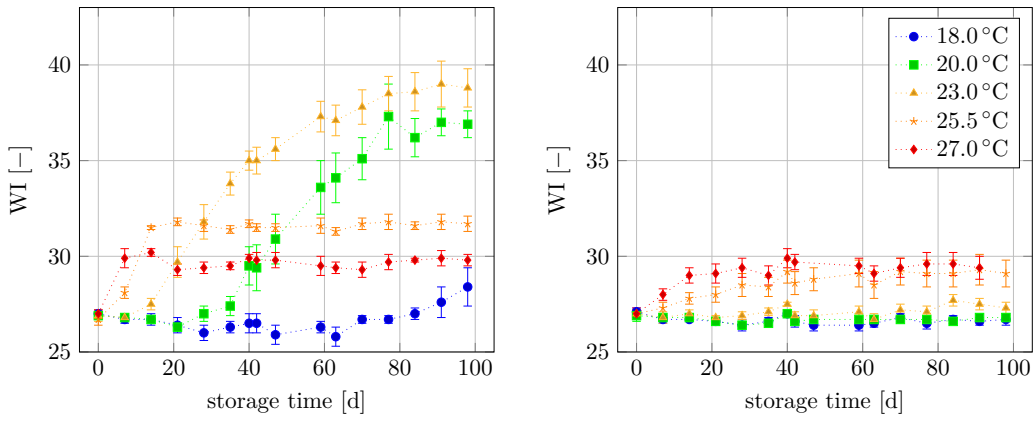


Figure 4.27: SFC of emptied chocolate shells obtained from pralines, which were subject to isothermal or cycling storage with intervals of 3 d and 7 d for 28 d in total

4.4.1.2 Whiteness index (WI) as indicator for fat bloom formation

The WI can be used as indicator for fat bloom formation, as described in chap. 3.6.7 and shown by Schütz et al. 2016. Fig. 4.28a and 4.28b show the WI increase over time for filled pralines and chocolate shells stored at isothermal temperatures.

It can be seen that WI increase of samples starts earlier at higher storage temperatures. In contrast, WI after 98 d storage is highest for filled pralines stored at 23 °C. Chocolate shells also show a WI increase during storage, especially at higher temperatures. The picture of a filled praline after 98 d storage, shown in fig. 4.29, suggests that the WI increase of filled pralines stored at high temperatures is not only an indicator for fat



(a) filled pralines (b) chocolate shells

Figure 4.28: WI of isothermally stored filled pralines and chocolate shells in dependency of storage time

bloom, but also for color change. The filled praline, which was stored at 20 °C shows a whitish, rough surface with a typical fat bloom pattern. The praline stored at 27 °C shows a yellowish color with a regular and smooth looking surface. This means that the WI is an indicator for fat bloom, but an additional visual assessment is necessary.

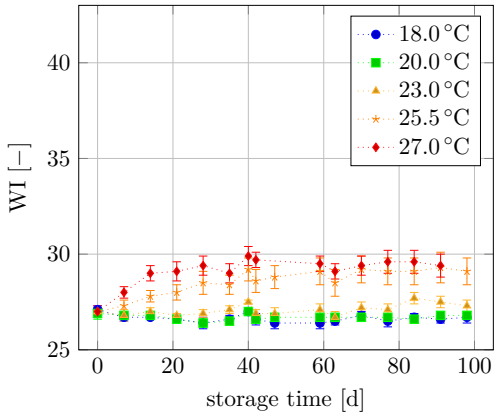
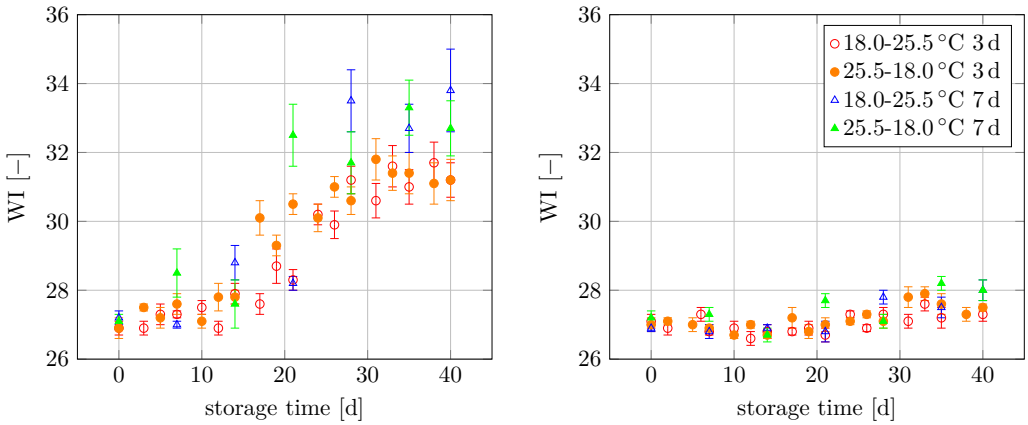


Figure 4.29: View of the top of a fresh praline and after 98 d isothermal storage at 20 °C and 27 °C

Pralines stored at cycling temperatures show alternating high-low or 'zig-zag' curve progression of the WI during storage of the samples, which can be seen in fig. 4.30a for filled pralines and in fig. 4.30b for chocolate shells. This is in contrast to the continuous curve progression, which was observed for isothermally stored filled pralines. The starting temperature of pralines subjected to cycling temperature storage had an impact on the start of the WI increase. Filled pralines, which were first stored at the higher tem-

perature showed an earlier WI increase. Regarding the cycling frequency, filled pralines with weekly cycling interval reached higher WI values after two to three weeks of storage than those treated with a cycling interval of 3 d.



(a) filled pralines (b) chocolate shells

Figure 4.30: WI of filled pralines and chocolate shells, which were subject to cycling temperature storage in dependency of starting temperature, second temperature, cycling frequency in days and storage time

4.4.1.3 Discussion

Isothermal storage of filled chocolate products at high temperatures is an easy and defined way for accelerated storage tests. However, the optimum temperature for fat bloom formation has to be identified. Storing at too high temperatures facilitates fast oil migration from the filling into the shell. At the same time, high temperatures impede fat crystallization at the surface. In contrast the crystallization intensity is higher at lower temperatures, while the oil migration rate from the filling into the shell is low (Ziegleder and Schwingshandl 1999).
SFC decreases linearly with an increasing amount of hazelnut oil in chocolate (see chap. 4.8). The results show that SFC of emptied shells decreases during storage for all pralines (see fig. 4.25 and 4.26), which confirms that the amount of hazelnut oil increases. With isothermal storage at 25.5 °C and 27.0 °C, saturation relative to concentration is reached after about 30 d, while for pralines stored at lower temperatures further migration can be assumed (see fig. 4.25). Reaching saturation at cycling temperature storage takes more than 40 d (see fig. 4.26).
The mean storage temperature of cycling storage is 21.75 °C, which was none of the isothermal storage temperatures but is in between 20.0 °C and 23.0 °C. However, the SFC decrease of emptied shells from pralines, which were subject to cycling storage temperatures is much stronger than that of pralines subjected to isothermal storage at 20.0 °C or 23.0 °C. This could be caused by the negative-exponential dependence of

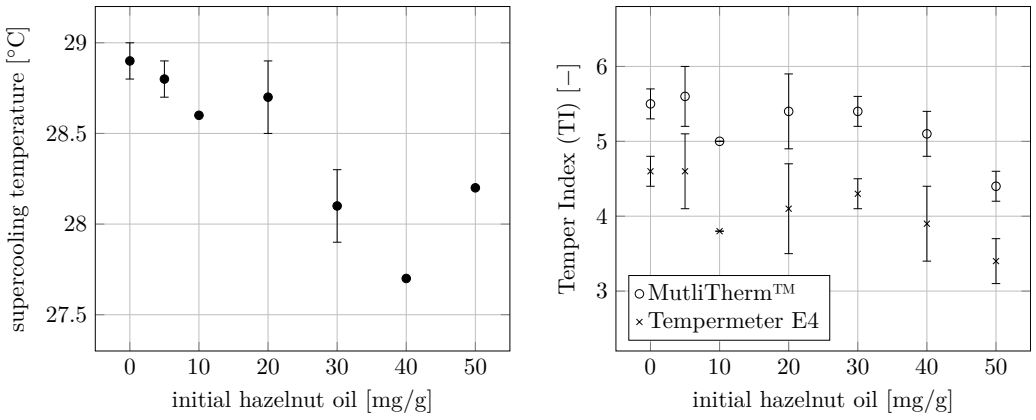
migration and storage temperature. The low migration rate during storage at the lower cycling storage temperature cannot compensate the high migration rate at the higher storage temperature.
The noticeably low SFC of emptied shells from pralines subjected to cycling storage temperatures, as can be seen in fig. 4.26, which were first stored at 25.5 °C for 3 d, indicates that additional changes occur. During the first high-temperature storage period, a high amount of hazelnut oil migrated, which softens the chocolate shell and might reduced resistance to further migration. During the second low temperature storage period, the previously melted fat in the sample can crystallize again. During this recrystallization, capillaries may form which facilitate further migration.
Crystallization is promoted at lower temperatures in the range of 15 °C to 18 °C, especially in the presence of crystals which can act as nuclei (Zeng 2000). This also occurs at the praline surface and the formed crystals become visible as fat bloom.
Fat bloom is indicated by a WI increase during storage, which starts earlier for pralines stored at higher isothermal temperatures. For low storage temperatures a decrease of the WI can be observed in the beginning (see fig. 4.28). This is caused by a loss of gloss. Due to the glossy surface, light is reflected and captured as white spots on the surface. These spots increase the lightness (L^*-value) of the whole sample. After a few weeks of storage at low temperatures the pralines turn matte and the light is not reflected any more, resulting in a lower L^*-value and thus in a lower WI (Briones and Aguilera 2005; Ziegleder and Mikle 1995b). At storage temperatures of 23 °C and above the fat bloom formation was too fast to observe this decrease and the WI increase started immediately. The WI increase of pralines stored at 25.5 °C and above was also observed in chocolate shells but to a lesser extent, as can be seen in fig. 4.28b. Thus the increased WI at higher storage temperatures might not only be caused by fat bloom formation but also by a color change of the chocolate (Briones and Aguilera 2005).
Even though the WI is affected by color change, it is much more effective for fat bloom evaluation than a panel. The costs are lower and the pictures can be evaluated using different criteria even after the storage test has ended. Additionally the constant lighting conditions reduce the deviation of the results.
For choosing an accelerated storage test several parameters have to be taken into account. Temperatures during storage and distribution might fluctuate and cycling storage tests seem close to reality. Yet cycling during distribution and storage includes a smaller temperature range and lower overall temperatures than tested in scientific studies. Independent of real conditions the results indicate, that even short temperature peaks cause earlier fat bloom. Cycling temperature storage at adequate levels includes the optimum temperature range for fat bloom formation, but takes crystallization and migration influence in unequal account and further structural changes cannot be excluded.
Isothermal storage and a maximum temperature are the target of most storage agreements. An accelerated storage test at isothermal conditions causes fast fat bloom formation and provides further information: relationship between temperature change, migration and crystallization can be described mathematically. However, several isothermal temperatures have to be investigated to find the optimum for fast fat bloom formation.

4.4.2 Simulating mixing during production

As was described by Rothkopf, Danzl, and Ziegleder 2016 filling fats and oils can be mixed into the chocolate during production unintentionally. A model system was used to study the effects of initial hazelnut oil in chocolate. For this purpose dark chocolate was mixed with hazelnut oil and cold stamping was used to produce praline shells which were filled with nougat and stored at different temperatures.

4.4.2.1 Observations during production

For praline production chocolate with initial hazelnut oil had to be tempered with a target temper index (TI) of 5.0, which is the optimum for standard dark chocolate according to industry. The supercooling temperature had to be reduced with increasing hazelnut oil content as known from experience. At the same time reaching the target TI of 5.0 measured using a Bühler MultiTherm™ and Sollich Tempermeter E4 became more difficult. The achieved TI and the corresponding supercooling temperature in the tempering machine can be seen in fig. 4.31a and fig. 4.31b.



(a) Supercooling temperature

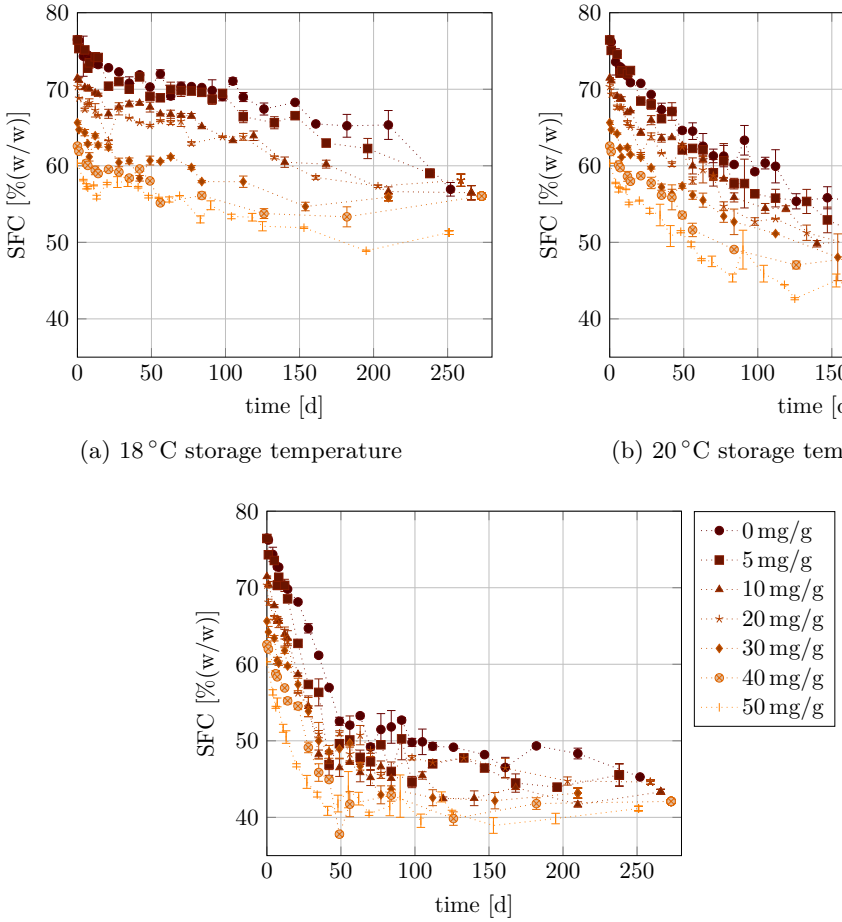
(b) Temper Index (TI)

Figure 4.31: Supercooling temperature and Temper Index (TI) measured using Bühler MultiTherm™and Sollich Tempermeter E4 in dependency of initial hazelnut oil in dark chocolate

4.4.2.2 Migration during storage

Hazelnut oil migration from the nougat filling into the shell chocolate was calculated by using the SFC measured via nuclear magnetic resonance (NMR) with structure preservation as described in chap. 3.6.2.1. The SFC during storage at 18 °C, 20 °C and 23 °C is shown in fig. 4.32 for each initial hazelnut oil concentration.

It can be seen that the SFC decreases faster at higher temperatures. The SFC of emptied shells from pralines stored at 23 °C proceeds towards saturation after about



(a) 18 °C storage temperature

(b) 20 °C storage temperature

(c) 23 °C storage temperature

Figure 4.32: SFC of emptied chocolate shells obtained from pralines stored at 18 °C, 20 °C and 23 °C and in dependency of the initial fraction of hazelnut oil

50 d of storage, while the SFC of emptied shells from pralines stored at 20 °C reaches saturation after around 200 d of storage and for emptied shells from pralines stored at 18 °C saturation was not reached during storage time.
There is a difference in the starting value of the SFC, which is lower in samples with a higher amount of initial hazelnut oil. This difference is constant during storage and corresponds to the reduction caused by hazelnut oil addition found in chap. 4.2.1. To visualize the impact of initial hazelnut oil on migration, the difference in the starting value was eliminated by using the ΔSFC, which is the difference between the SFC at storing time t and SFC_{start} at the beginning of storage ($t = 0$), as shown in eg. 4.4.1.

$$\Delta SFC(t) = SFC_{start} - SFC(t) \tag{4.4.1}$$

The ΔSFC over time is shown in fig. 4.33 for pralines with increasing amounts of hazelnut oil stored at 18 °C.

The ΔSFC increases during storage. The curve progression is steeper at short storage times and proceeds towards a first plateau at around 100 d of storage. After this period a steep increase of the ΔSFC can be observed, which indicates swelling. Thus, only the storage of up to 100 d was regarded, which is enlarged in fig. 4.33b.
It was assumed that a) the amount of migrated oil is similar for all samples at the end of the migration process, because the amount of filling and chocolate are the same, b) the specific length $l = \frac{A}{V}$ (see chap. 2.4.1) equals the shell thickness of $l = 3$ mm, c) the shell thickness is constant for all samples and d) according to chap. 4.2.1 a ΔSFC of 1 %(w/w) equals 5 mg/g oil in chocolate. The simplification of the solution of the second Fickian law, given in eq. 2.4.1, can than be written as eq. 4.4.2

$$m(t) = \frac{1}{l} \cdot \sqrt{D_{eff} \cdot t} \tag{4.4.2}$$

The linearization is plotted in fig. 4.34. A linear fitting was applied for the first 25 d of storage, shown in fig. 4.33c, because the migrated amount in the beginning is proportional to the square root of time.

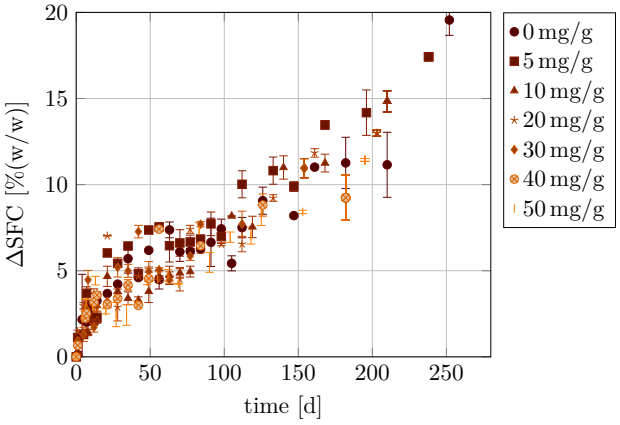
The resulting slope of the linearization $m_{lin} = \frac{\Delta m_t}{\Delta \sqrt{t}}$ can be used to calculate D_{eff} as shown in eq. 4.4.3.

$$D_{eff} = \left(\frac{\Delta m_t}{\Delta \sqrt{t}} \cdot l \right)^2 \tag{4.4.3}$$

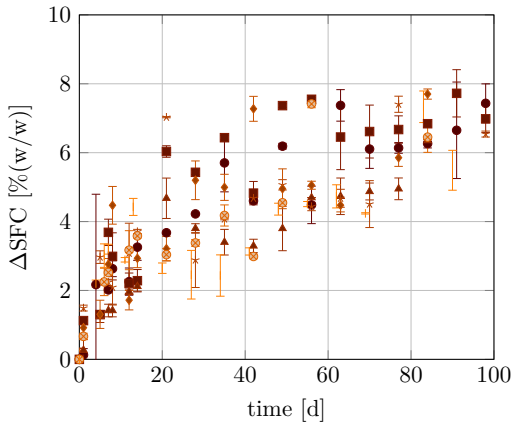
The values for D_{eff} for hazelnut oil migration in dark chocolate stored at 18 °C are listed in tab. 4.8. The D_{eff} for an initial fraction of hazelnut oil of 50 mg/g was regarded as an outlier. For the other concentrations, the resulting D_{eff} increases with higher amounts of initial hazelnut oil.

4.4.2.3 Polymorphic changes during storage

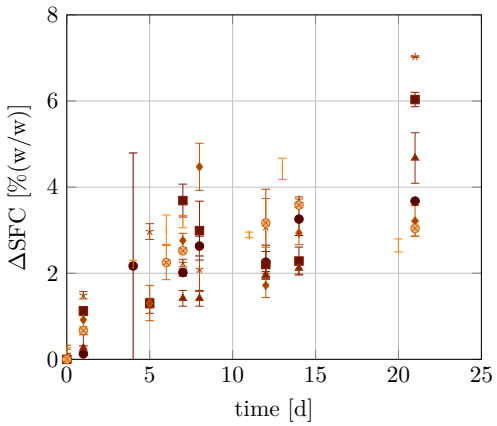
Hazelnut oil accelerates polymorphic transition of cocoa butter crystals (K. W. Smith, Cain, et al. 2007). Thus, melting curves of the emptied chocolate shells were recorded



(a) full storage time of 280 d



(b) enlarged section for the first 100 d of storage



(c) enlarged section for the first 25 d of storage

Figure 4.33: Δ SFC of dark chocolate nougat praline shells during storage at 18 °C, in dependency of the initial fraction of hazelnut oil

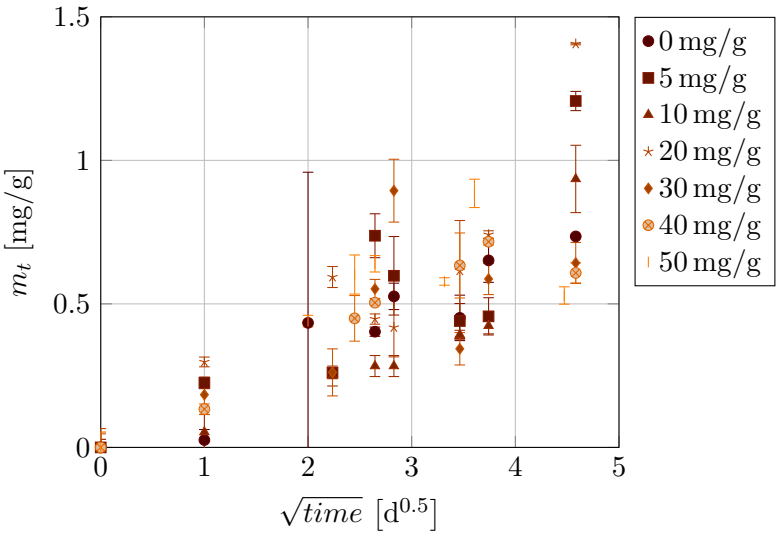


Figure 4.34: Migrated hazelnut oil during storage at 18 °C, for different amounts of the initial fraction of hazelnut oil

Table 4.8: D_{eff} calculated for hazelnut oil migration in dark chocolate pralines stored at 18 °C, in dependency of initial hazelnut oil with standard error and correlation coefficient R^2 of the linear regression

initial hazelnut oil mg/g	D_{eff} 10^{-15} m^2/s	R^2 -
0	1.8 ± 0.3	0.752
5	1.1 ± 0.7	0.175
10	27.5 ± 2.7	0.885
20	39.4 ± 8.3	0.652
30	53.4 ± 13.3	0.578
40	76.9 ± 8.5	0.881
50	0.1 ± 2.9	0.0002

via differential scanning calorimetry (DSC) according to chap. 3.6.3.1 during storage of the pralines. An increase of the melting temperature indicates a polymorphic transition from the β_V into the β_{VI} crystal form.
At the beginning of the experiments the samples were measured using the DSC auto-sampler. It was noticed that the auto-sampler storage plate gets warm during measurement. Further investigations revealed that temperatures of 26 °C to 28 °C are reached, which can causes partial melting of the samples prior to the measurement. This could be seen by noticeably low melting enthalpies and irregular curve shapes. Those samples were excluded from further evaluation. For the other samples the peak maximum temperature was evaluated. The results for pralines without initial hazelnut oil in the chocolate shell, which were stored at 18 °C, 20 °C and 23 °C are shown in fig. 4.35.

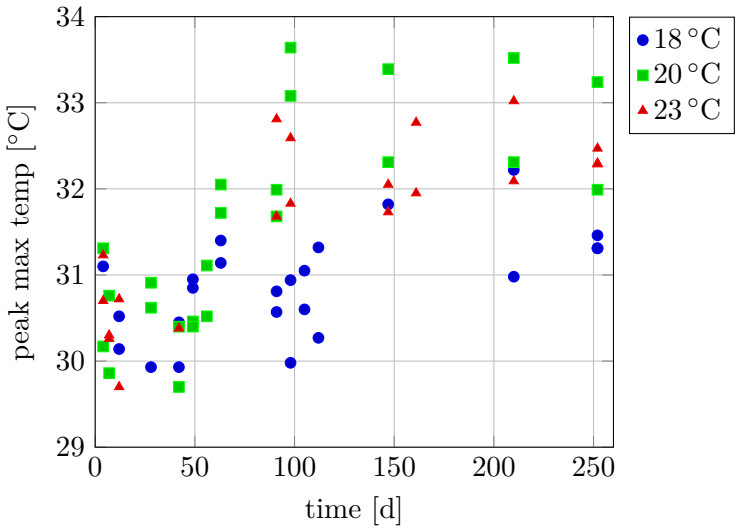


Figure 4.35: Maximum peak temperature of melting curves obtained via DSC indicating the polymorphic crystal form of chocolate from pralines without initial hazelnut oil, stored at 18 °C, 20 °C and 23 °C and shown as single values

During storage an increase in peak maximum temperature can be observed, which is linked to the formation of a more stable polymorphic crystal form (see tab. 2.7 in chap. 2.2.1). While the peak maximum temperature in the beginning is around 30 °C to 31 °C for all samples, which indicates a β_V polymorphic form, it increases and temperatures around 33 °C are reached, which indicate a β_{VI} polymorphic form. The peak maximum temperature increases earlier at higher storage temperatures.
The impact of the initial fraction of hazelnut oil in the chocolate shell on the maximum melting peak temperature was compared for pralines stored at 20 °C. The results are shown in fig. 4.36.

The high variation of the measured temperatures can be explained by the transition from the β_V into the β_{VI} crystal form, which occurs in clusters and not uniformly distributed throughout the sample.

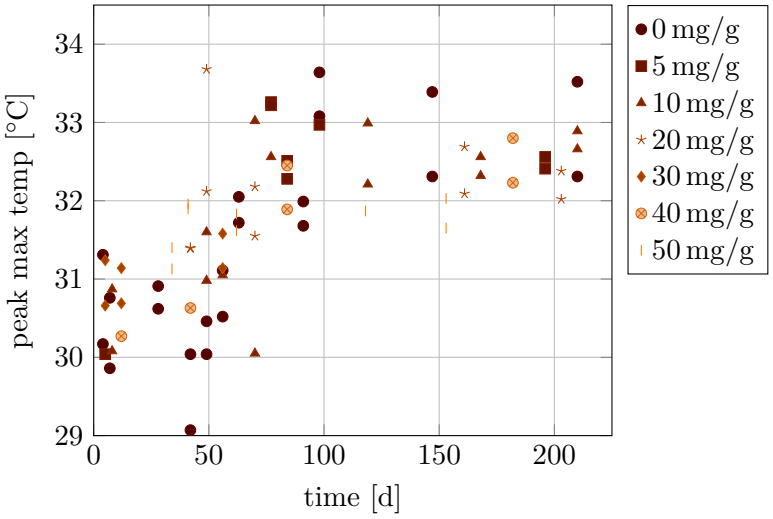


Figure 4.36: Maximum peak temperature of melting curves obtained via DSC for emptied shells of pralines stored at 20 °C with increasing amounts of initial hazelnut oil, measured in duplicate and shown as single values

The peak maximum temperature of pralines with 50 mg/g initial fraction of hazelnut oil in the shell rise above a melting temperature of 31.5 °C first and that of pralines without an initial fraction of hazelnut oil increases at last. That confirms the finding of K. W. Smith, Cain, et al. 2007 that the transition from β_V to β_{VI} polymorphic crystal form is accelerated in the presence of hazelnut oil, which again accelerates migration.

4.4.2.4 Fat bloom development during storage

The dependency of fat bloom development measured using the WI on storage conditions was shown and discussed in chap. 4.4.1. Filled pralines were most stable at an isothermal storage temperature of 18 °C and at a storage temperature of 23 °C the strongest fat bloom formation could be observed. Accordingly fat bloom development at 18 °C was used to investigate differences in start of fat bloom formation. Fat bloom development at 23 °C, shown in fig. 4.37, was used to investigate differences in maximum fat bloom.

The WI is slightly lower for pralines with a higher initial fraction of hazelnut oil and the further curve progression also depends on this initial fraction of hazelnut oil in the following way. In the beginning the WI decreases for samples with a lower initial fraction of hazelnut oil. For pralines without hazelnut oil the WI decreases from about 29 to 26, while pralines with 50 mg/g initial fraction of hazelnut oil decrease from 27.5 to 26.5. After decreasing the WI increases during further storage. However, the saturation value is lower for samples with higher amounts of initial hazelnut oil, but the quality limit is already exceeded clearly before, according to Schütz et al. 2016.

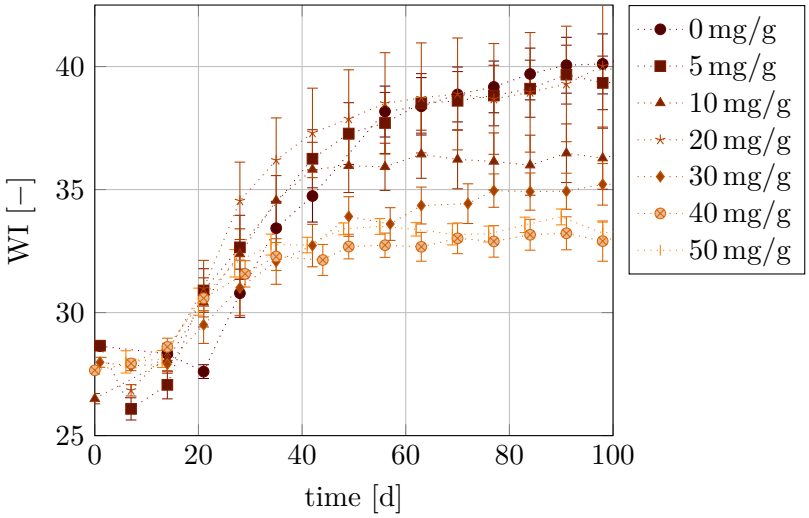


Figure 4.37: WI of dark chocolate nougat praline shells during storage at 23 °C and in dependency of the initial fraction of hazelnut oil

4.4.2.5 Discussion

During storage of the filled pralines the oil migration, polymorphic transition of the crystal form and fat bloom development were investigated. The starting hypothesis was, that initial hazelnut oil causes a lower degree of crystallization of the chocolate, which facilitates migration of hazelnut oil during storage. On the other hand, initial hazelnut oil reduces migration pressure due to a lower concentration difference and it reduces the SFC. Chocolate with a lower SFC is reported to be less resistant to migration (Motwani et al. 2011). However, the amount of migrated hazelnut oil indicated by SFC is only slightly depending on the initial content of hazelnut oil.
To directly compare the migration in samples with different amounts of initial hazelnut oil , the effective diffusion coefficient D_{eff} was calculated for each content of initial hazelnut oil. The D_{eff} were similar to those found by Silva et al. 2007, who investigated migration of diphenylbutadiene (molar mass 206 g/mol) from plastics into chocolate and found an effective diffusion coefficient of 29×10^{-15} m^2/s for chocolate stored at 25 °C. The values are in a similar range, even though the molar mass is different. The molar mass of hazelnut oil can be estimated from the main components triolein (OOO) and trilinolein (LLL) with molar masses of 885 g/mol and 879 g/mol, respectively. A similar diffusion coefficient of substances with four times higher molar masses indicates that there are other processes beside diffusion such as capillary flow.
However, higher amounts of migrating hazelnut oil are supposed to accelerate the polymorphic transition (K. W. Smith, Cain, et al. 2007). This can be partially confirmed, since pralines stored at higher temperatures include more migrated hazelnut oil and show earlier polymorphic transition. However, this polymorphic transition might also be accelerated by the increased temperature itself. Comparing pralines with increasing

amounts of initial hazelnut oil at the same storage temperature, polymorphic transition occurs earlier in samples with higher amounts of initial hazelnut oil, which can be seen by the temperature increase in fig. 4.36. The difference in double determination is caused by the irregular transition of polymorphic forms. The transition is supposed to start in small clusters and spreads throughout the chocolate. The small amount of sample used for DSC, which was around 10 mg, might be taken from a cluster, where the transition already startet or from a spot of the sample, where the transition did not start. It is not possible to see the clusters before the sampling.
Migration and polymorphic transition are supposed to accelerate fat bloom formation (Ziegleder, Geier-Greguska, et al. 1994). However, the WI indicated, that pralines with an initial fraction of hazelnut oil are less affected by loss of gloss. Visual investigation revealed, that pralines with higher amounts of initial hazelnut oil are less glossy and have a lighter color. The glossy surface is a sign of properly tempered chocolate with a high amount of crystallized fat (Juul 2010). Initial hazelnut oil might have disturbed crystal formation and growth during manufacturing of the praline, as was shown in chap. 4.1.2. Thus, insufficient crystallization of the chocolate before removing from the molds might result in a less glossy surface. This will be part of further research.
The impact of a initial fraction of hazelnut oil could also be observed during production, where a lower supercooling temperature was needed for chocolate tempering. Additionally, this was also observed in chap. 4.1 for DSC cooling curves with increasing amounts of vegetable fats. Chocolate with 50 mg/g initial hazelnut oil had a TI below 5. However, the supercooling temperature was too high compared to the other chocolates with initial hazelnut oil. Thus, it cannot be excluded that the effects observed for samples with 50 mg/g initial hazelnut oil are caused by insufficient tempering.
The crystallization process of fat bloom at the surface is also affected by hazelnut oil, resulting in slower crystallization and less crystallized fat as to be seen in chap. 4.1.2. After the start of fat bloom development further fat bloom growth is indicated by a WI increase. The rate of fat bloom growth and the saturation value are lower for pralines with increasing amounts of initial hazelnut oil. This might also be explained by the reduced crystallization rate caused by the initial fraction of hazelnut oil.
Initial hazelnut oil has several effects in filled dark chocolate nougat pralines. During production the crystallization rate and the amount of crystallized fat is reduced. This could also be observed for palm oil and hazelnut oil mixtures (Hubbes, Braun, et al. 2020b) and results in less resistance against migration. On the other hand, the already present hazelnut oil is supposed to reduce migration pressure but the effects seem to cancel each other out. Further, hazelnut oil is known to promote polymorphic transition (K. W. Smith, Cain, et al. 2007), but elevated storage temperatures also accelerate polymorphic transition as well as migration of hazelnut oil. Thus the identification of the cause in pralines where migration occurs is not possible. The effect of initial hazelnut oil can likewise not be clarified, since several effects are overlapping.

4.4.3 Post-treatment of pralines

Crystallization can be divided in nucleation, crystal growth and post-crystallization. During post-crystallization the crystal amount is increased, the crystal network gets denser and more stable. Thus, fully crystallized chocolate should be more resistant against migration, if no defects, e.g. cracks, are formed. The idea of post-treatment of chocolate is to accelerate post-crystallization (Juul 2010). Therefore freshly cold stamped pralines were taken from the praline plant before the first cooling tunnel and the filling step and were subjected to post-tempering. To identify optimal temperatures for post-tempering, a simulation of the process in the DSC was done. For this purpose tempered chocolate was taken directly from the tempering machine and placed in an aluminum pan, which was put directly in the DSC and measurement was started. The time and temperature protocol was described in chap. 3.5.4.1 and is shown in fig. 4.38.

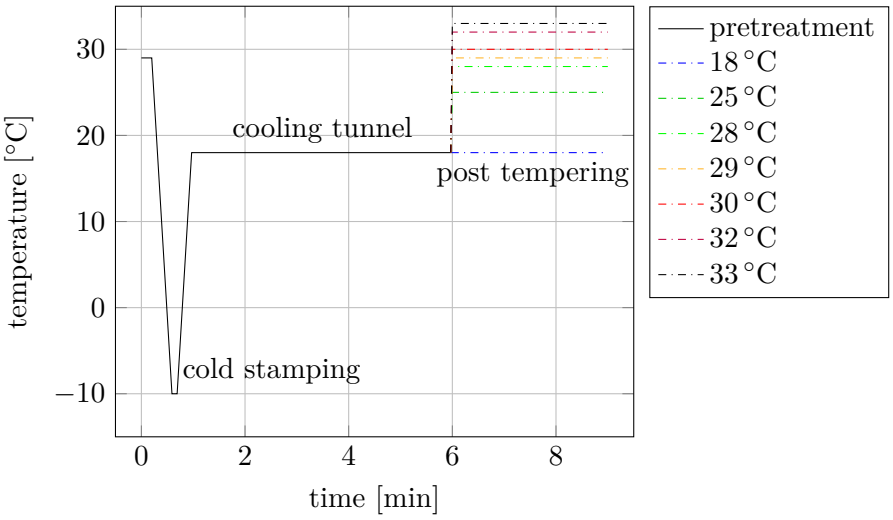


Figure 4.38: Time-temperature profile of the simulated post tempering DSC process

The subsequent melting curve gives information about the amount and polymorphic form of crystallized fat and is shown in fig. 4.39 for different post-tempering temperatures.

A clear shift of the peak maximum temperature can be seen, when post-tempering is performed at 28 °C to 30 °C. Therefore these temperatures were chosen for praline production. Since the chocolate volume of pralines is much bigger than the chocolate volume in a DSC pan, the holding time was increased from 3 min to 15 min. The holding time for the praline shells was determined based on experimental data such as DSC melting curves. During storage, the SFC of the emptied shells was determined to quantify hazelnut oil migration and is shown in fig. 4.40 for pralines stored at 20 °C.

Post-tempering has a slight impact on the SFC starting value and the slope of SFC over time remains constant. The SFC progression over time for pralines made of shells which were subject to post-tempering at 30 °C show a high standard deviation compared

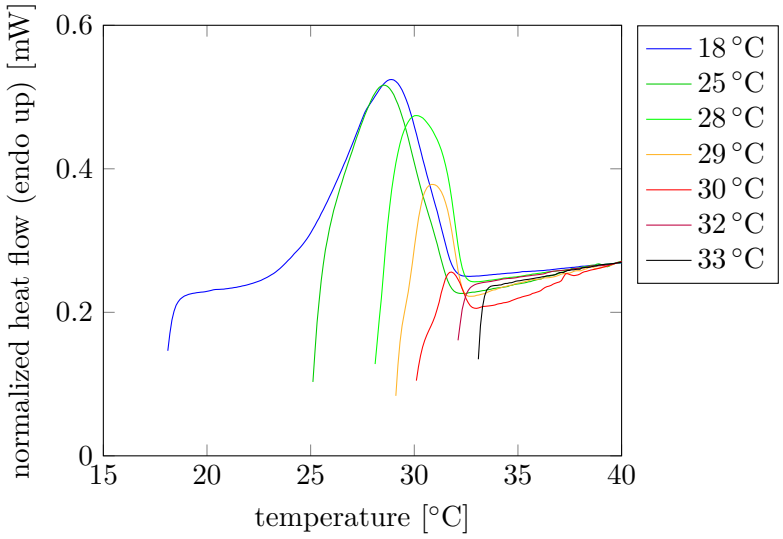


Figure 4.39: Melting curves of tempered chocolate after process simulation in a DSC with varying post-tempering temperatures

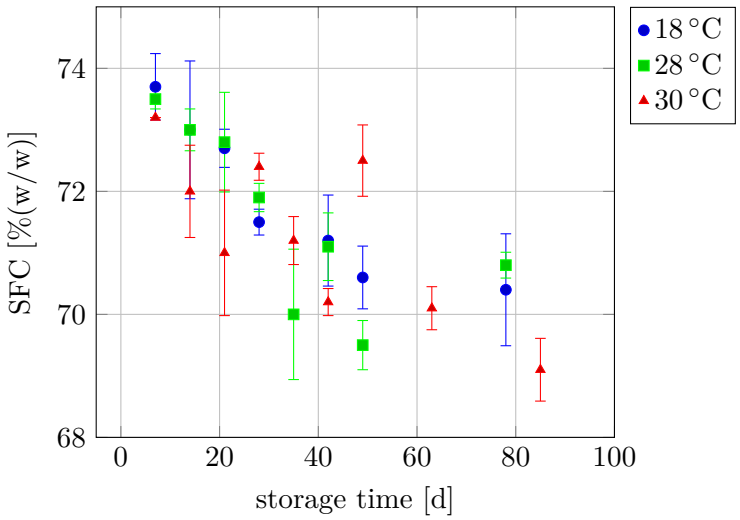


Figure 4.40: SFC of pralines without post-tempering at 18 °C and with post-tempering at 28 °C and 30 °C for 15 min followed by subsequent storage at 20 °C measured with β-stabilization in duplicate

to those pralines, which were subject to non post-tempering at 18 °C and post-tempering and 28 °C. This can also be observed for peak maximum temperatures of DSC melting curves, shown in fig. 4.41, which were recorded to observe polymorphic change.

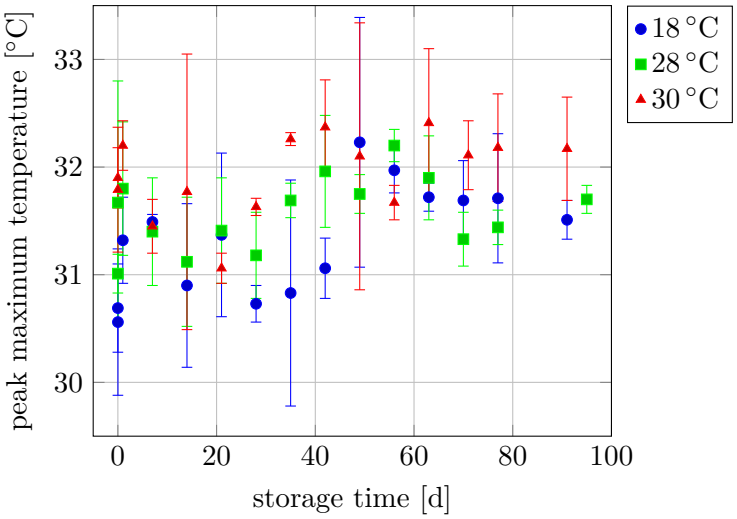


Figure 4.41: Peak maximum temperature as indicator for the polymorphic crystal form of melting curves of pralines without post-tempering at 18 °C and post-tempered at 28 °C and 30 °C stored at 18 °C determined in duplicate

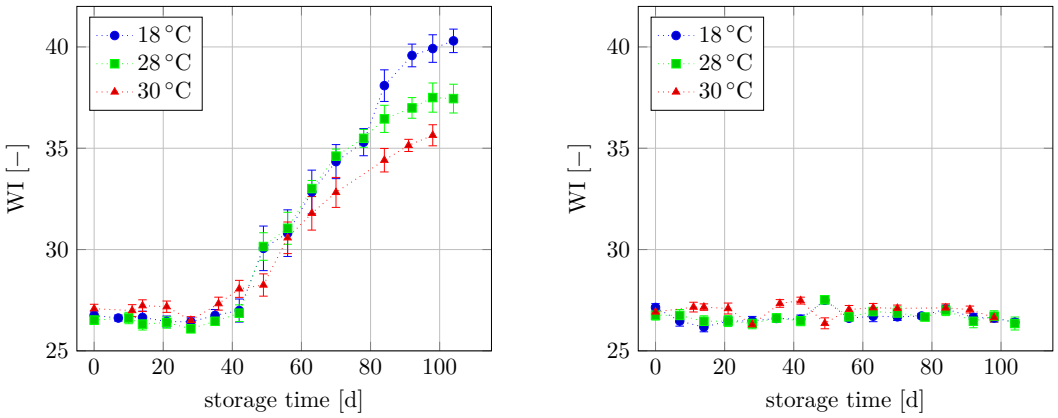
The peak maximum temperature of melting curves from pralines made of shells which were subject to post-tempering at 30 °C show high standard deviations, as could also be observed for the SFC values. These pralines also show higher peak maximum temperatures and the earliest increase in peak maximum temperature, which is an indicator for polymorphic transition. Since polymorphic transition is a known factor for fat bloom formation, the WI was recorded during storage and is shown in fig. 4.42.

Start of fat bloom formation, which is indicated by WI increase, seems to be independent of post-tempering. On the contrary, the WI after 100 d of storage is lower for pralines, which were subject to higher post-tempering temperatures. The WI of pralines without filling remained at constant low values.
The optical appearance of the pralines with and without post-tempering differed. In fig. 4.43 pictures of fresh pralines after 7 d storage and of bloomed pralines after 49 d storage at 23 °C with and without post-tempering are shown.

Pralines, which were subject to post-tempering at 30 °C show an even surface with less fat bloom, compared to pralines without post-tempering.

4.4.3.1 Discussion

Post-tempering of pralines might be a way to accelerate post-crystallization and reduce quality loss of filled chocolates. Simulating post-tempering using DSC showed, that only high-melting fractions remain in the chocolate. However, too high temperatures lead to



(a) filled pralines

(b) empty shells

Figure 4.42: WI of pralines and empty shells, which were produced without post-tempering at 18 °C or with post-tempering at 28 °C and 30 °C and subsequent storage at 20 °C

(a) Non-tempered pralines after storage for 7 d at 23 °C

(b) Post-tempered pralines after storage for 7 d at 23 °C

(c) Non-tempered pralines after storage for 49 d at 23 °C

(d) Post-tempered pralines after storage for 49 d at 23 °C

Figure 4.43: Dark chocolate nougat pralines stored at 23 °C without (left) and with shell post-tempering at 30 °C (right)

complete melting of the crystals.
Post-tempering at adequate temperatures around 28 °C to 30 °C had an impact on visual appearance of the pralines. There is no clear impact on migration, but pralines, which were subject to post-tempering at 30 °C had high standard deviations regarding SFC values as well as melting peak maximum temperatures obtained via DSC. This indicates that the pralines have been subject to partial melting, which might have accelerated crystal transformation. The pralines made from shells which were subject to high post-tempering temperatures had higher DSC melting peak maximum temperatures, which indicates higher melting crystal fractions. Additional to the higher peak maximum temperatures in the beginning, pralines made from shells which were subject to high temperature post-tempering showed earlier increase of peak maximum temperatures indicating a polymorphic transition.
However, the start of fat bloom formation, indicated by WI increase is not affected by post-tempering, while the maximum fat bloom intensity is. In contrast, the pictures of the pralines showed, that the fat bloom is not evenly distributed. The results show that chocolate stabilization prior to filling increases fat bloom stability.

5 Summary

Fat bloom is a major topic for quality assurance of filled chocolate products. Migration of oil from the filling into the chocolate is supposed to be the main trigger. Therefore, most studies focus on the migration of oils from the filling into the chocolate. In the underlying migration mechanism, both diffusion and capillary flow are discussed.
However, the impact of crystallization on migration and resulting fat bloom is rarely investigated. The crystalline state of cocoa butter in chocolate has a major impact on chocolate microstructure, which influences migration during storage. The interaction of migration and crystallization is complex, but their understanding is mandatory for high-quality products with long shelf-life.
The aim of this thesis was to identify the interaction of crystallization of the fatty phase and migration of oil from the filling into the surrounding chocolate in filled dark chocolate products and their impact on fat bloom formation. Therefore analytical methods for quantification of filling oils in chocolate had to be established and the impact of migration on crystallization as well as of crystallization on migration had to be evaluated in model systems. Afterwards a realistic praline was produced and the interaction of migration and crystallization was evaluated.
Analyzing crystallization of cocoa butter was of special interest to identify the impact of vegetable oil addition on it. The effect of vegetable oil addition on cocoa butter crystal growth depends on the triacylglyceride composition with regard to number of double bonds and chain length of the fatty acid. All added vegetable oils reduced the speed of crystallization and diluted the samples, which caused reduction of the relative amount of crystallized fat. This effect showed a linear correlation to the concentration of the added oils. Thus, measuring the amount of crystallized fat could be used to quantify the amount of vegetable oil in cocoa butter and chocolate.
The influence of the crystal state on migration was less pronounced. The migration in samples with β_{VI} crystal forms was significantly accelerated compared to samples with β_{V} crystal forms. However, the polymorphic transition from β_{V} to β_{VI} crystal forms was not sufficiently accelerated by the added oils or the particles present in the samples to significantly increase oil migration in industrial products.
The findings in this study show that the crystalline state of cocoa butter in chocolate is crucial for migration of oils from the filling into the chocolate. This crystalline state is influenced by initially present oils from the filling, which might be introduced during production. The migration of oils from the filling into the chocolate can not be investigated without considering the crystalline state of the chocolate. However, the mechanism of migration in chocolate, diffusion or capillary flow, could not be conclusively clarified, but the hypothesis of a combination of the mechanisms is supported.
Migration or unintentional addition of vegetable oils or fats to the chocolate during

production results in a lower solid fat content of the chocolate, which was supposed to accelerate migration during storage. However, the microstructure of chocolate with a lower amount of crystallized fat showed less resistance against migration, but the migration rate was reduced. On the other hand migration during storage accelerated crystal transition from β_V to β_{VI} in the solid state.
The practical application of this thesis mainly focuses on possible methods to reduce fat bloom caused by migration. Post-treatment by heating the shell before filling the pralines resulted in a stable and fully crystallized praline with a glossy surface. Initial hazelnut oil in the chocolate shell caused slower crystallization and a lower final solid fat content. Thus, pralines with initial hazelnut oil are less resistant against migration of oil from filling into the chocolate and show a less glossy surface. However, fat bloom appeared later during storage, but already the surface of fresh pralines might not be acceptable for the producers and customers.
Beside the newly found importance of the crystalline state of cocoa butter in chocolate, it could be seen that the polymorphic transition of crystals from β_V to β_{VI} massively accelerates migration, but is too slow in real products to affect industrial production.
This work also revealed more findings to be addressed in future research. Throughout the whole study, the filling base consisted of the same nougat. However, the mobility of the oil in the filling is a limiting factor for migration, since bound or immobilized oil can not migrate. The currently used models for migration assume an infinite oil resource. Additionally the contact area between the filling and the chocolate are of interest, because an air gap might act as a boundary for migration.
In this study, questions concerning the influence of the chocolate and its crystalline state on migration of oil from the filling into the chocolate in filled chocolate products such as pralines could be clarified. However, the influence of the oil content and mobility in the filling and the contact area between filling and chocolate should be addressed in future research.

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