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ORIGINAL PAPER

Impact of Limonene on the Physical Properties of Reduced Fat Chocolate

T.-A. L. Do · J. Vieira · J. M. Hargreaves · B. Wolf · J. R. Mitchell

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Abstract The addition of limonene, a low molecular weight hydrophobic compound, to chocolate was reported to decrease the hardness and the viscosity of chocolate, facilitating the production and improving the eating quality of reduced fat chocolate. The objective of this study is to understand the functionality of limonene in decreasing the viscosity and the hardness of chocolate, a fat (cocoa butter)-based particulate suspension. This study shows that chocolate hardness was decreased because limonene mixes with cocoa butter, affects its crystallization pattern and decreases its solid fat content. After checking that limonene does not significantly affect the continuous phase volume fraction, we show that limonene decreases chocolate viscosity by decreasing the viscosity of the continuous phase, cocoa butter. The addition of low quantities of limonene in cocoa butter leads to a great decrease in the liquid fat viscosity. The dependence of the viscosity on the ratio of cocoa butter to limonene analyzed using Kay's equation seems to indicate that limonene mixes with and within the cocoa butter triglycerides, diluting the fat and leading to a decrease in the overall fat viscosity.

Keywords Viscosity · Crystallization · Chocolate · Cocoa butter · Limonene

Introduction

The development of a reduced fat chocolate with no compromise in eating quality is a response to consumer demand for healthier diets. There are, however, some technical issues that must be overcome before this can be successfully achieved.

In terms of microstructure, chocolate is a suspension of sugar, cocoa solids and milk solids, dispersed in a fat continuous phase based on cocoa butter. As it is desirable for particle sizes to be below 30 μm to prevent gritty texture, a fat content of approximately 30%wt is usually required to coat the surfaces of all the particles. Therefore, the fat content in regular chocolate usually ranges between 30 and 40%wt. The chocolates studied in this research have a total fat content of 25%wt. Reducing the fat content in chocolate causes a significant increase in the molten chocolate viscosity, because there is less fat to coat the particles, reducing the particles' ability to flow past each other. The thickening of molten chocolate is an issue in its processing as it makes operations like pumping, molding or enrobing more difficult. The sensory properties are also affected. Reduced fat chocolates have been reported to be harder, to exhibit poor melting properties and to be difficult to swallow [1, 2]. It appears that decreasing the viscosity of reduced fat chocolates may also help overcome the reduction in these sensory qualities.

Several approaches that can be used to fluidize molten reduced fat chocolates have been reported; these include optimizing the particle size distribution [3, 4], using fat replacers [1], replacing cocoa butter by a water-in-oil emulsion [5], and increasing emulsifier levels or using emulsifier blends [6]. In addition to these methods, a patent published in 1999 by Beckett [1] claims that the addition of limonene to molten chocolate in quantities of up to 5%

T.-A. L. Do (✉) · B. Wolf · J. R. Mitchell
Division of Food Sciences Sutton Bonington Campus,
School of Biosciences, The University of Nottingham,
Loughborough, Leicestershire LE12 5RD, UK
e-mail: linedo@hotmail.com

J. Vieira · J. M. Hargreaves
Nestlé Product Technology Centre York, PO Box 204,
Haxby Road, YO91 1XY York, UK

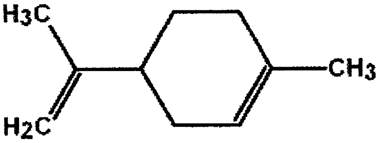

Fig. 1 Chemical structure of limonene

leads to a significant decrease in the chocolate viscosity, therefore facilitating the production of reduced fat chocolates. The resulting chocolates were also found to be softer and to melt more easily in the mouth compared to a chocolate with a similar fat level of pure cocoa butter and without limonene.

Limonene is a terpene, see Fig. 1, that exists in D- and L-forms. It is naturally present in the D-form in various essential oils and is frequently found in food products, generally as a flavor compound. Chocolates containing limonene have an orange flavor.

Although the benefits of adding limonene to reduced fat chocolates have been reported, the functionality of limonene remains unclear. The aim of this study is to understand the underlying principles of the action of limonene in decreasing the viscosity and the hardness of chocolate. Since limonene is a hydrophobic material existing in the form of liquid oil, it can be assumed that it mixes with the cocoa butter, affecting its physical properties, such as its viscosity and crystallization pattern. These modifications are suspected to lead to the changes observed in chocolate viscosity and hardness, both of which are strongly linked to chocolate texture.

To test this hypothesis, chocolates containing limonene and control samples without limonene were manufactured; all with the same total continuous phase content of 25%wt. Chocolate viscosity and hardness were measured instrumentally. Additionally, relevant physical properties such as viscosity, density, solid fat content (SFC) and melting profile were determined for the continuous phases of the two chocolates, solely cocoa butter or a mixture of cocoa butter and limonene. The action of limonene was also compared to those of milk fat and hazelnut oil, which are two fats/oils sometimes used in chocolate formulations and which are known to affect the softness of chocolate.

Experimental Procedures

Chocolate Recipes and Manufacture

For the purpose of this study, a milk chocolate basis with a total fat content of 22.3%wt was manufactured in a pilot plant. Initially, all ingredients, see Table 1, were mixed in a blender (K175, Crypto Peerless Ltd., Birmingham, England) to produce a gross premix. Then, this premix was refined to particle sizes of below 30 μm using a three-roll refiner (Buhler Ltd., Uzwil, Switzerland). Afterwards, the refined mix was conched for 4 h at 50 °C in a conch (Lipp Mischtechnik GmbH, Mannheim, Germany), and then split into four batches of 1 kg. To each batch, previously heated to 50 °C, cocoa butter, anhydrous milk fat, hazelnut oil or limonene was added in an amount corresponding to 3%wt of the total chocolate mass or 13%wt of the total continuous phase content. The mixture was then homogenized for 30 min at room temperature using a kitchen blender (N50 Stand Mixer, Hobart, Troy, OH, USA). After storage overnight at 50 °C, the chocolates were hand-tempered and set in cylindrical plastic moulds (diameter: 10 mm; height: 12 mm) followed by a storage at 10 °C for 2 h before de-moulding.

This procedure allowed the manufacture of four chocolates with the same continuous phase weight fraction (25.3%wt) and the same particle size distribution but varied continuous phase composition; see Table 1 for recipes. Values for the total fat content are also provided. It should be noted that although limonene is a hydrophobic compound, it is not counted as fat.

Chocolate Viscosity

The shear viscosity of the chocolates was determined at 40 °C using a controlled-stress rheometer (Bohlin CVO100, Malvern Instruments, Malvern, UK) fitted with a concentric cylinder geometry (bob diameter 25 mm, cup diameter 27.5 mm). The following procedure was applied: after a pre-shear period of 5 min at 50 s^{-1}, the shear rate was increased from 2 to 50 s^{-1} in 3 min, then maintained for 1 min at 50 s^{-1}, before finally being decreased from 50 to 2 s^{-1} in 3 min. Shear stress and instantaneous viscosity were recorded and plotted against the shear rate. This procedure is a modified version of the standard method recommended for conventional chocolates by the International Confectionary Association (ICA), previously the International Office of Cocoa, Chocolate and Confectionery (IOCCC) [7]. The modification in this study consists of pre-shearing at 50 s^{-1} instead of 5 s^{-1}, which was found to remove the time dependency initially observed on our reduced fat samples. As recommended by the ICA and applied in the chocolate industry, the yield stress was taken as the shear stress at 5 s^{-1}, and the apparent viscosity as the viscosity at 40 s^{-1}, both on the ascending shear rate ramp.

Viscosity of Fats

Fat mixtures based on cocoa butter only and mixtures of cocoa butter with milk fat, hazelnut oil or limonene of the

Table 1 Chocolate recipe

Steps	Ingredients	Trade name	Content (%wt)
Common milk chocolate basis (22.3% fat)	Sugar	Granulated sugar (British Sugar, Peterborough, UK)	49.8
	Cocoa powder (11% fat)	Natural cocoa powder 10/12 (De Zaan, Koog aan de Zaan, Netherlands)	9.0
	Skim milk powder	Spray-dried SMP (Dairy Crest Ingredients, Surrey, UK)	17.0
	Cocoa butter	Cocoa butter (ADM, Hull, England)	20.5
	Soy lecithin	Soy lecithin, GMO-free (Nestlé UK, York, England)	0.3
	Polyglycerol polyricinoleate	PGPR (Nestlé UK)	0.4
Subsequent addition of fat (+3%)	Cocoa butter	Cocoa butter (ADM)	3.0
	Or milk fat	Anhydrous milk fat (Corman, Goé, Belgium)	
	Or hazelnut oil	Hazelnut oil (Tesco, Cheshunt, UK)	
	Or limonene	Deodorized D-limonene (FD Copeland & Sons Ltd., London, England)	
			Total fat content (%wt) = 25.3 or 22.3 (for limonene)

same composition as in the chocolates (87:13) were prepared. To further investigate the impact of limonene on the viscosity of the fat phase, additional blends (cocoa butter: limonene) were prepared (25:75), (50:50), (75:25), (95:5). Fat phase viscosities were measured at 40 °C, the same temperature as applied to the chocolates, using the same rheometer as above fitted with a larger cylinder geometry to increase measurement sensitivity (bob diameter: 33.5 mm, cup diameter: 37.5 mm). An increasing shear stress ramp was applied logarithmically from 0.01 to 1 Pa and steady state viscosities were recorded. The viscosity of the fat blends was found to be Newtonian and the results reported corresponded to the mean value of the viscosity between 0.1 and 1 Pa s.

Density

The densities of the limonene, fats and binary mixtures were measured at 40 °C using a densitometer (DMA 5000, Anton Paar, Graz, Austria). As the measurements were reproducible within 0.1%, only duplicate measurements were conducted, and results are expressed as the mean value of two measurements.

Chocolate Hardness

Chocolate hardness was measured instrumentally using a texture analyzer (TA.XT Plus from Stable Micro Systems Ltd., Surrey, UK) at room temperature. The chocolate samples were set in a cylindrical shape with a diameter of 10 mm and a height of 12 mm. A cylindrical flat-ended stainless steel probe with a diameter of 2 mm was programmed to penetrate the chocolates prepared above to a depth of 5 mm at a speed of 1 mm s^{-1}. The trigger force was set to 0.05 N. Hardness was taken as the maximum peak force in Newtons. Results for hardness are expressed as the mean value of five replicates conducted on different samples.

Solid Fat Content

The SFC of the samples was determined using nuclear magnetic resonance (NMR). To study the SFC of the chocolates, or rather their respective fat phases, fats blends of the same composition as present in the chocolates were prepared and heated to 50 °C. The fat blends were seeded to ensure a similar crystallization as in tempered chocolate: after cooling the fat blends to approximately 32 °C, commercial cocoa butter seed crystals of the desired form (V or β_2) (Mycryo cocoa butter, Barry Callebaut, Lebbeke-Wieze, Belgium) were added to induce fat crystallization in the stable form. The pre-crystallized fat blends were then poured into NMR glass tubes with an internal diameter of 7 mm, filled to a height of 45 mm, which were subsequently placed into a water bath at 10 °C for 30 min to allow them to set and reach equilibrium. Without any further pretreatment, the SFCs of the fat blends were quantified at 15, 20, 25, 30 and 35 °C. Upon each temperature increase, the samples were allowed to equilibrate for exactly 30 min. The SFC measurements were then carried out on an NMR (Maran, Oxford Instruments, Abingdon, UK), using a commercial program supplied by the NMR supplier (SFC Direct software). The direct NMR method (AOCS Official Method CD16B-93) was used to measure and calculate SFC, after calibrating the apparatus using three standards of known SFC (0, 30 and 70% SFC). Once the temperature equilibrium was achieved, a pulsed NMR field of 24.4 MHz was applied, using a 90° pulse of approximately 4 µs. The SFC is determined by measuring the NMR signal at 11 and 70 µs after the initial pulse, respectively, giving a solid + liquid signal (S + L) and a liquid signal only (L). SFC determination is based on direct ratio measurement between the solid and liquid parts of the sample observed in the NMR free induction decay, and was calculated as:

$$\mathrm{SFC} = 1 - 100 \times \frac{L}{S + L}$$

where S is the signal amplitude due to the solid component and L is the amplitude of the signal from the liquid component.

Differential Scanning Calorimeter Measurements

Thermograms of the chocolates were established using a DSC (DSC-7, PerkinElmer, Waltham, MA, USA) in order to quantify their melting enthalpies and their melting peak temperatures. Approximately 5 mg of chocolate, previously flaked using a razor blade, were placed into a 30 µL DSC aluminium pan and hermetically sealed. Each sample was then equilibrated at 10 °C for 10 min before being subjected to a temperature scan between 10 and 50 °C at a rate of 5 °C min^{-1}. An empty pan was used as reference and the baseline was calibrated using indium. The results are presented as heat flow per g of sample (normalized heat flow). Melting enthalpy (peak area) and peak temperature were determined using the measurement software of the DSC (Pyris), and results are expressed as means of triplicates. Additionally, a crystallinity index at 20 °C (CI) was calculated as:

$$\mathrm{CI} = \mathrm{SFC}_{\mathrm{CB},20^{\circ}\mathrm{C}} \times \frac{\text{Melting enthalpy}}{\text{Melting enthaply}_{\mathrm{CB}}}$$

where $\mathrm{SFC}_{\mathrm{CB},20\ ^{\circ}\mathrm{C}}$ is the SFC of cocoa butter at 20 °C, as measured by NMR; $\mathrm{SFC}_{\mathrm{CB},20\ ^{\circ}\mathrm{C}} = 80\%$.

Results and Discussion

Viscosities of the Chocolates

The viscosity curves acquired for chocolates are displayed in Fig. 2. Each curve, distinguished by different symbols, has an upper part, corresponding to the increasing shear rate ramp, and a lower part, corresponding to the decreasing shear rate ramp. The difference between the upper and lower parts of the viscosity curve, known as thixotropy or time dependency, is minimal but indicative of the presence of particle aggregates in the chocolate. A more unusual observation here is that all chocolates exhibit a certain degree of apparent shear thickening, indicating that some particle aggregates might also build up as the shear rate increases. This effect has not yet been reported. It is likely to be linked to the high polyglycerol polyricinoleate (PGPR) content (0.4%), as such behavior is not observed in chocolates without or with low levels of PGPR (results not shown). PGPR is an emulsifier that greatly decreases and sometimes even removes chocolate yield stress [8, 9]. PGPR seems to be efficient at dispersing particle aggregates at rest or at low shear rates, but at higher shear rates the effect may be reversed at high levels of PGPR. Additional work is required to fully interpret the observation made here.

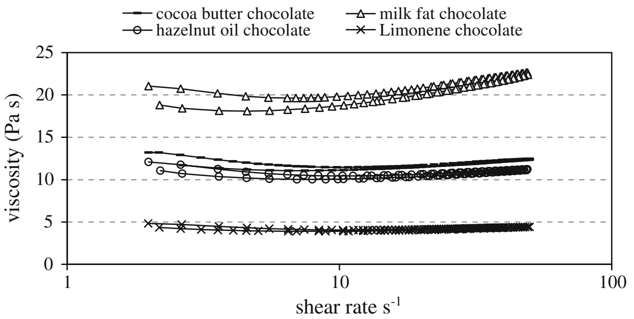

Fig. 2 Viscosity curves of chocolates with different continuous phase compositions

Values for the apparent viscosity and yield stress for each chocolate are reported in Table 2. The values for the yield stress correlate linearly to the apparent viscosity ($R^2 = 0.99$), which has been reported previously [3, 10]. The following discussion focusses on the apparent viscosity data, since the viscosity is a critical parameter in most unit processes, and is certainly critical in the design of reduced fat chocolates.

The results shows that replacing a fraction of the cocoa butter by limonene in a 25%wt fat chocolate leads to a significant decrease in the chocolate viscosity, supporting the patent claim. The viscosity of the chocolate containing limonene is significantly lower than the viscosities of the other chocolates investigated. It is around 4 Pa s, compared to 11–12 Pa s for the chocolates based on cocoa butter only and on cocoa butter with hazelnut oil. The chocolate containing milk fat displays a much higher viscosity, as reported previously [11]. This has been explained by a competitive adsorption between the added emulsifiers (lecithin and PGPR) and the free fatty acids (FAs) naturally present in the milk fat at the sugar surface. FAs have been shown to disperse sugar particles in fats in a less efficient way than lecithin or PGPR. Therefore, when present in

Table 2 Apparent viscosities and yield stresses of the chocolates

Chocolate continuous phase	Cocoa butter	Cocoa butter + milk fat	Cocoa butter + hazelnut oil	Cocoa butter + limonene
Apparent viscosity (Pa s)	11.8 ± 0.5	22.9 ± 3.0	11.1 ± 0.4	4.1 ± 0.2
Yield stress (Pa)	57 ± 3	100 ± 20	54 ± 2	20 ± 2

chocolate formulations, FAs adsorb at the sugar surface in competition with lecithin and PGPR, leading to a higher chocolate viscosity overall.

The viscosity reduction when using limonene is considerable. In fact, it renders the viscosity of 25%wt fat chocolate indistinguishable from the viscosity of a 30%wt fat chocolate.

This behavior can be understood by analyzing the viscosity data of the different continuous phases. The viscosity of a particulate suspension η_s depends mainly on the viscosity of the continuous phase, η_c, but also on the particle volume fraction, ϕ, and the intrinsic viscosity, $[\eta]$, as indicated by Krieger and Dougherty in the following equation [12]:

$$\eta_s = \eta_c \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta]\phi_m} \qquad (1)$$

Density could also have had an impact, since all of the chocolates in this study had the same continuous phase mass fraction. Having unequal densities for limonene and cocoa butter can affect the continuous phase volume fraction $(1-\phi)$ and therefore the particle volume fraction ϕ, which directly affects the viscosities of the suspensions, as Eq. 1 shows.

Densities of Fats and Fat Mixtures

The densities of the fats and the binary mixtures corresponding to the compositions of the different continuous phases are shown in Fig. 3. The densities of cocoa butter, milk fat, nut oil and their mixtures are in the range of 0.900 ± 0.005 g cm^{-3}. The density of limonene, however, is lower, around 0.840 g cm^{-3}, which certainly explains why the cocoa butter–limonene mixture also has a slightly lower density, 0.886 g cm^{-3}, than the other binary mixtures. The experimental value for the mixture density fits well with the density estimated from a model [13], previously shown to accurately predict the density of ideal mixtures. The density is calculated as the sum of the density of each pure component times the mole fraction. Based on this finding it can be suggested that cocoa butter and limonene form ideal mixtures, i.e. mixing is driven by entropy and there is little or no interaction between the two components.

The density of the cocoa butter–limonene mixture is lower than the density of cocoa butter, but actually it hardly affects the continuous phase volume fraction, which only increases by $\frac{25\%}{0.886} - \frac{25\%}{0.895} = +0.3\%$vol. This difference in continuous phase volume fraction is too low to explain the observed decrease in the chocolate viscosity, which is from 12 to 4 Pa s. As mentioned earlier, the viscosity of the limonene chocolate is equivalent to the viscosity of a 30%wt fat chocolate. Therefore, the order of magnitude decrease in chocolate viscosity observed could only be explained by an increase in continuous phase volume fraction of at least 5–6%vol, assuming, of course, that there is no change in the viscosity of the continuous phase.

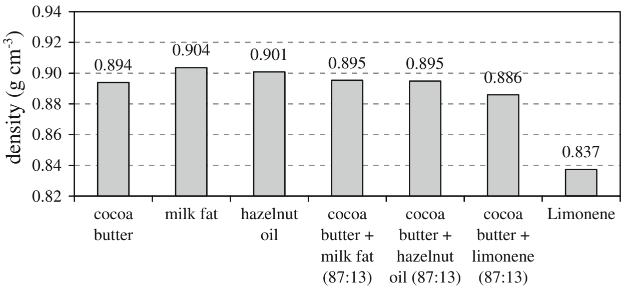

Fig. 3 Density of fats and fat blends corresponding to the continuous phase composition

Viscosities of Fat Mixtures

The viscosities of the fat mixtures corresponding to the different chocolate continuous phases are given in Fig. 4.

The continuous phases containing cocoa butter only and binary mixtures of cocoa butter–milk fat and cocoa butter–nut oil have viscosities of approximately 50 mPa s, whereas the viscosity of the cocoa butter–limonene mixture is much lower, around 26 mPa s. This result indicates that the limonene affects the chocolate viscosity by reducing the viscosity of the continuous phase. This hypothesis is also reinforced by the fact that the viscosity ratio of the

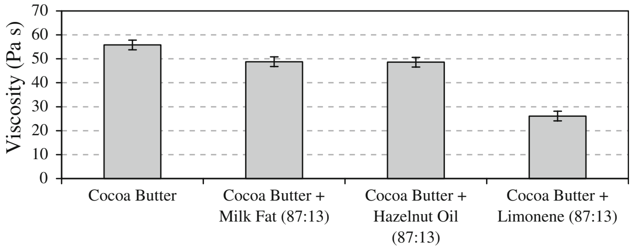

Fig. 4 Viscosities of the continuous phases

chocolate with cocoa butter to the chocolate with limonene is fairly close to the viscosity ratio of the cocoa butter to the cocoa butter–limonene mixture (87:13), 2.1 and 2.8, respectively. According to Eq. 1, the ratios should be equal.

Viscosities of Cocoa Butter–Limonene Mixtures

The viscosity decrease caused by the addition of limonene to cocoa butter has not yet been reported in the literature, probably because a mixture of fat (cocoa butter) with a low molecular weight hydrophobic compound (limonene) in such a ratio (87:13) is not often encountered in food formulations, or has not caused any noticeable viscosity issues. Fats and oils are mainly composed of triacylglycerides (TGs), molecules consisting of three free FAs esterified on a glycerol backbone. The viscosity of fats is an important parameter in the design of unit processes and has been extensively studied. It is well known that the viscosity of oils and fats results from the molecular interactions between the long-chain glyceride molecules. The viscosity increases with increasing TG molecular weight but decreases with unsaturation and temperature [14–17]. This observation is supported by the hypothesis that, in a liquid oil, the TGs might not be randomly dispersed but specifically organized in a bilayer lamellar structure [18, 19]. This structure is likely to be related to the need to accommodate the three hydrocarbon chains in a space-filling arrangement [18]. The shape and size of the lamellar structures changes with the diffusion rate of the molecules. Thus, the oil viscosity is an indirect measurement of the degree of structure of the TGs in the liquid state [18–20]. Various attempts have been made to predict the viscosity of fats; parameters used include temperature [17, 21, 22], number of carbons (or molecular weight) and degree of unsaturation [23, 24], FA chain lengths [25], composition of FAs [26] or composition of TGs with temperature [15], etc. Previous studies have shown that the addition of a lower molecular weight TG or a lower molecular weight and less viscous hydrophobic material to a given fat or heavy oil leads to a decrease in the mixture viscosity [15, 16, 27–29]; this property is for example used to fluidize biodiesels [25, 30]. It appears that limonene decreases the cocoa butter viscosity in a similar fashion. It can be hypothesized that limonene modifies the native bilayer lamellar TG structures in the molten cocoa butter, leading to the development of smaller bilayers. This would decrease the aliphatic chain interactions, accounting for the fat viscosity. There may be a minor interaction between the TG and the limonene, but this may not be detected with the density measurements. Structural changes are often detected early as changes in the rheological behavior.

More clues to the cocoa butter–limonene mixture behavior can be obtained by comparing the experimental viscosity with the theoretical values. It has previously been reported that Kay's equation [2] can be utilized to estimate the viscosity of oils. Kay's equation estimates the viscosity of a mixture as a function of the viscosity of pure components (TGs) and the mole fraction [15]:

$$\ln(\eta_{\text{mix}}) = \sum x_i \ln \eta_i \tag{2}$$

where x_{i} is the mole fraction and η_{i} is the kinematic viscosity of a pure component.

Kay's mixing rule implies that the natural logarithm of the viscosity of a mixture is a combination of the natural logarithm of the viscosity of each initial material. Therefore, the viscosity decrease caused by the addition of limonene may simply be a result of increasing the amount of a low-viscosity component in the simple mixture.

An indication that cocoa butter–limonene mixtures do not behave as simple mixtures is given by the fact that Kay's equation does not properly describe the mixture viscosity, as

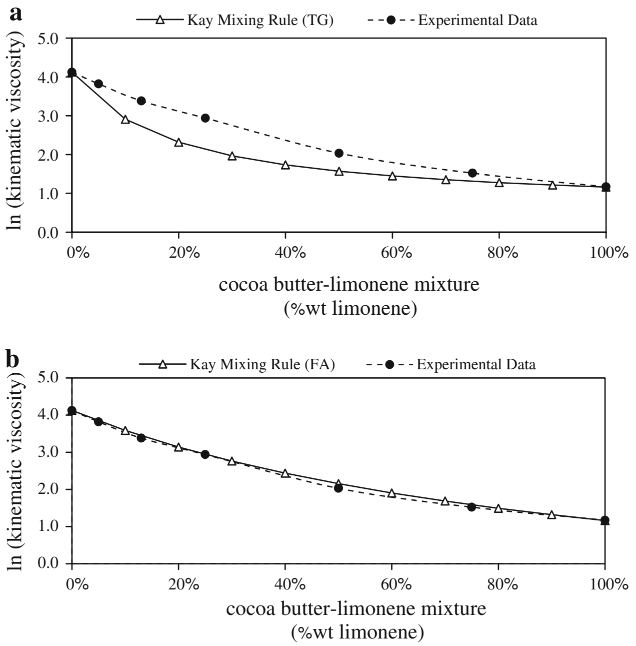

Fig. 5 **a** The kinematic viscosity of cocoa butter and limonene blends in various ratios, where the *dashed line* with the *dark filled circles* represents experimental data, and the *continuous line* with *open triangles* shows the estimated values from Kay's mixing rule, obtained using molar masses of 136 g mol^{-1} for limonene and of 853 g mol^{-1} for cocoa butter TG; see the calculation in Table 3. Kinematic viscosities were calculated as the ratios of dynamic viscosity to calculated density; see Table 3 for density values. **b** The kinematic viscosities of cocoa butter and limonene blends in various ratios, where the *dashed line* with *dark filled circles* represents experimental data, and the *continuous line* with *open triangles* depicts values estimated with Kay's mixing rule, using molar masses of 136 g mol^{-1} for limonene and 270 g mol^{-1} for cocoa butter fatty acids (*FA*), see the calculation in Table 3. Kinematic viscosities were calculated as the ratios of dynamic viscosity to calculated density; see Table 3 for density values

indicated by Fig. 5a. For viscosity estimation, the mole fraction of cocoa butter was calculated from the cocoa butter TG composition found in the literature [31], see Table 3. Figure 5A shows that the experimental values for the cocoa butter–limonene mixture kinematic viscosity are higher than the theoretical values obtained with Kay's equation.

The deviation from Kay's mixing rule is hypothesized to arise from steric effects, which occur when mixing materials with different molecular volumes and sizes such as limonene and TGs. With their aliphatic chains of 16 to 18 carbons, cocoa butter TGs are much bigger molecules than limonene. In addition to having molecular weights that are about six times higher than that of limonene, the TGs have a specific open configuration, arranged in a chair-shaped conformation [19]. The mixing of limonene with cocoa butter may occur not only at the limonene–TG level but could also at the FA level. This hypothesis is reinforced by the fact that Kay's equation perfectly fits the experimental data if the molar mass of the cocoa butter FAs (and not TG) is considered; see Fig. 5B. Data in Fig. 5b suggest that the cocoa butter–limonene mixture does not behave as a TG–limonene mixture but rather as a FA–limonene mixture. The fact that the glycerol backbone ties three FAs together in the TG may not influence the rheology of the mixture to any major extent. Limonene may get in-between the FA chains in the TGs so that the TGs behave like individual FAs. This result suggests that limonene may be mixing not only with but also within the TGs. The effect of limonene on the fat viscosity is likely to be a simple dilution effect, with limonene possibly inserting between the TGs and also within the FAs of the same TG.

Hardnesses of the Chocolates

As outlined in the "Introduction", one of the textural characteristics of chocolate that is altered by reducing fat content is hardness. Beckett claims that the addition of limonene decreases the hardness of chocolate [1], which is confirmed by the data of this study; see Fig. 6.

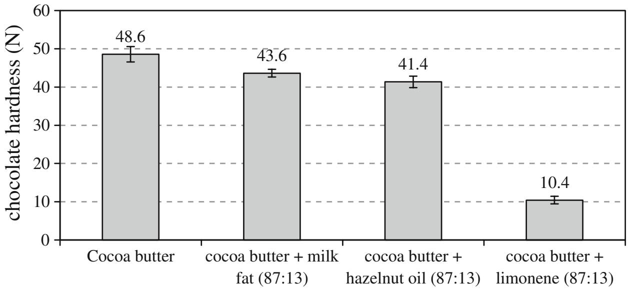

Fig. 6 Hardnesses of different chocolates at 20 °C

Results clearly demonstrate that the chocolate based solely on cocoa butter is the hardest, with an instrumental hardness of approximately 50 N. Chocolates containing a fraction of milk fat or hazelnut oil are softer, as shown by the decrease in hardness to about 40 N. Strikingly, the chocolate containing the same fraction of limonene exhibits a much lower hardness, around 10 N. It appears that limonene has a pronounced softening effect on chocolate compared to milk fat or nut oil. This is beneficial for the texture, as limonene renders reduced fat chocolates as soft as full fat chocolates (32.5%wt fat), which typically show a hardness of around 15 N using the same measuring technique [2].

The softening effect of limonene on chocolate can be understood by analyzing the SFC. The presence of liquid in the crystallized fat matrix softens the chocolate [32, 33]. In the case of reduced fat chocolates, their increased hardness is mainly due to the reduction of the overall fat content and, therefore, the liquid fat content. There could also be an increased particle interaction effect that would make the chocolate more resistant to breakage, and thus harder [3].

Solid Fat Content

The effect of limonene on the SFC of cocoa butter is shown in Fig. 7. The effect of limonene is compared to those of milk fat and hazelnut oil. Additionally, thermograms for the chocolates are displayed in Fig. 8.

Table 3 Cocoa butter composition according to Karlshamns' *Handbook of Vegetable Oils and Fats* [31], and calculations of cocoa butter TG molar mass and cocoa butter (CB) FA molar mass

Chain length	Formula	Molar mass (g mol^{-1}) = $12N_C + 16N_O + N_H$	Relative content of FA in CB (%wt)	Molar mass fraction of the FA in CB (g mol^{-1}) = (molar mass) × (relative content)
C16:0	$C_{16}H_{32}O_2$	256.42	26	66.67
C18:0	$C_{18}H_{34}O_2$	284.47	36	102.41
C18:1	$C_{18}H_{34}O_2$	282.46	33	93.21
C18:2	$C_{18}H_{30}O_2$	280.44	3	8.41

Average molar mass of a cocoa butter fatty acid (FA) = 270.70 g mol^{-1} = Σ(molar mass fraction of the FA)

Average molar mass of a cocoa butter TG = 853.18 g mol^{-1} = 3 × average FA molar mass (270.70 g mol^{-1}) + glycerol molar mass (41.08 g mol^{-1})

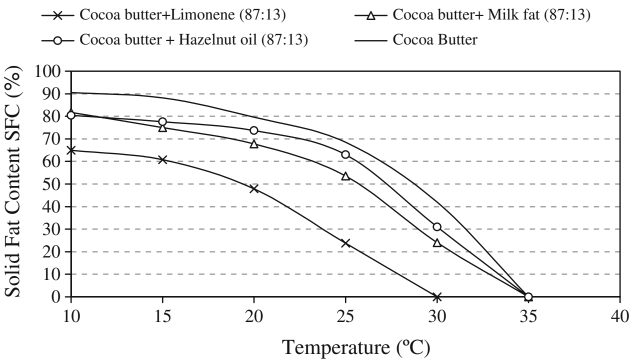

Fig. 7 Solid fat contents of the chocolate fat phases at different temperature

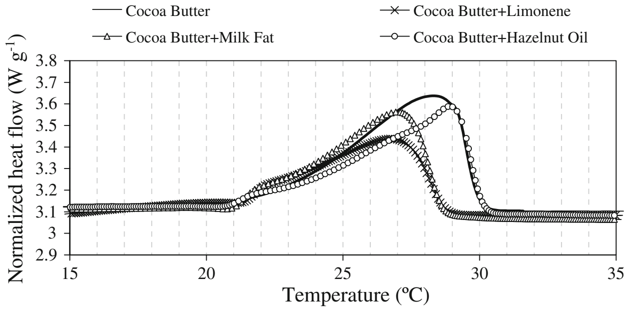

Fig. 8 Melting DSC thermograms of the different chocolates (increasing temperature scan)

Figure 7 shows that the SFC of cocoa butter decreases upon the addition of a fraction of milk fat, hazelnut oil or limonene over the whole temperature range. The addition of milk fat or hazelnut oil decreases the SFC of cocoa butter to a comparable degree (15–25%), with the addition of milk fat having a larger effect. However, the addition of the same fraction of limonene to cocoa butter leads to a much greater decrease in the SFC (over 50% above 25 °C). There is a strong positive linear correlation between chocolate hardness and measured SFC at 25 °C ($R^2 = 0.94$), supporting the hypothesis that limonene softens chocolate by decreasing the SFC of cocoa butter.

DSC Measurements

As seen in Fig. 8 and Table 4, including milk fat, hazelnut oil or limonene in the chocolate leads to a decrease in fat crystallinity, which explains the observed decrease in hardness. The addition of limonene reduces the formation of fat crystals most significantly; the fat crystallinity drops from 80% for cocoa butter-based chocolate down to 55% for the limonene chocolate. It is worth noting that SFC measurements provide a slightly lower value for the amount of fat crystal formed, around 48%. This trend has also been observed previously [34].

A comparison of the melting temperature ranges for the different crystalline phases indicates that the onset melting temperature is hardly affected. However, a decrease in the end melting temperature (T_{end}) is observed with the addition of limonene or milk fat to cocoa butter. T_{end} is around 30 °C for cocoa butter and nut oil chocolates and is reduced to about 29 °C for the limonene and milk fat chocolates. This suggests that the decrease in crystallinity could result from a decrease in the amount of fat crystals with higher melting temperature, or in a decrease in crystal perfection and size. X-ray diffraction analysis should provide further information on the crystal type and size formed.

In chocolate, limonene could affect many features in the crystallization of cocoa butter. First, limonene could modify the crystallization pattern of cocoa butter by causing crystal lattice imperfections. Limonene could be incorporated into the lattice and may alter the subsequent structure, as was also suggested for impurity molecules [35]. Such effects have already been reported with milk fat TGs, which are markedly different in size and shape to those of cocoa butter [36]. The same could be true and may even be emphasized with limonene, the structure of which is totally different from the one of a TG. It also appears from a previous study [20] that the organization of the TGs in the liquid fat before crystallization will affect some crystallization parameters, such as the nucleation temperature and the crystal growth rate. As mentioned earlier, limonene may dilute cocoa butter TGs and modify the native bilayer lamellar TG structures in the cocoa butter melt. Therefore, limonene could also affect the whole process of cocoa butter

Table 4 Melting enthalpies, crystallinity indices and characteristic melting temperatures of the different chocolates

Chocolate fat composition	Melting enthalpy (J g^{-1} of chocolate)	Crystallinity index at 20 °C (%)	Onset melting temperature (°C)	Melting peak temperature (°C)	End melting temperature (°C)
Cocoa butter	28.6 ± 0.3	80	20.6 ± 0.2	28.0 ± 0.6	30.3 ± 0.6
Cocoa butter + milk fat	23.7 ± 0.3	66	21.5 ± 1.0	27.3 ± 0.7	28.9 ± 0.5
Cocoa butter + hazelnut oil	25.7 ± 0.2	71	20.6 ± 0.3	28.8 ± 0.1	29.3 ± 0.5
Cocoa butter + limonene	19.8 ± 1.8	55	20.6 ± 0.3	26.7 ± 0.6	30.1 ± 0.2

crystallization, including the nucleation stage and the crystal growth. As a result, the overall microstructure of the fat crystal network, influenced by the cluster size and number, may also change. Based on the crystallinity indices, the amount of crystalline phase is reduced from 80% for cocoa butter to 55% with the addition of 13% of limonene to cocoa butter. Since limonene does not crystallize at the temperature range investigated, calculations indicate a mixture crystallinity of around 70% $[\approx 80\%(100 - 13\%)]$, as opposed to the measured value of 55%. This result suggests that limonene has prevented the crystallization of approximately 20% $\left(\approx \frac{70-55\%}{70\%}\right)$of the cocoa butter TGs. Limonene may act as a physical barrier to the packing and arrangement of TG chains leading to crystallization.

A decrease in crystallinity is often observed for miscible blends and can result from two causes. This happens first in the case of incompatible fats, when the TGs of two fats impede the crystallization of each other. A mixture that causes such a softening effect is called eutectic; a typical example is the mixture of cocoa butter and milk fat [35]. The decrease in crystallinity is also observed when two compatible fats with very different melting points are blended, such as cocoa butter and peanut oil. In this latter case, the softening effect is dilutional [35]. A combination of these two effects may be happening in the case of cocoa butter–limonene mixtures. The dilutional effect is likely to occur since limonene does not crystallize at the temperature range investigated. Additionally, it appears that limonene prevents the crystallization of cocoa butter, and it is possible that limonene is incorporated into a fat crystal and affects the crystallization pattern.

In this study, the functionality of limonene in decreasing the viscosity and the hardness of chocolate was determined. It appears that limonene mixes with the cocoa butter in chocolate and affects cocoa butter viscosity and crystallization. The addition of small amounts of limonene to cocoa butter leads to a significant decrease in the liquid fat viscosity. Limonene seems to mix with and within cocoa butter TGs, diluting cocoa butter and leading to a decrease in the overall viscosity. It also appears that the presence of limonene in cocoa butter affects cocoa butter crystallization, reducing the amount of fat crystals formed. A reduced SFC was observed when limonene was added to cocoa butter. This study demonstrates that the viscosities of liquid fat or liquid fat-based dispersions can potentially be controlled by adding a low molecular weight hydrophobic compound such as limonene, which dilutes the TGs. The principles elucidated in this study are expected to be applicable beyond the reduction of fat content in chocolate. This study provides important information on the partitioning of low molecular weight compounds in fat matrices and on how it can be used to control the viscosities of liquid fats.

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