



Influence of ethylcellulose—Medium chain triglycerides blend on the flow behavior and β -V polymorph retention of dark chocolate



Marcelo R. Ceballos^a, Karina L. Bierbrauer^{a,b}, Sonia N. Faudone^a, Silvia L. Cuffini^c, Dante M. Beltramo^{a,b,d}, Ismael D. Bianco^{a,b,e,*}

^a Centro de Excelencia en Productos y Procesos de Córdoba (CEPROCOR), Argentina

^b Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

^c Instituto de Ciência y Tecnologia, Universidade Federal de São Paulo, Brazil

^d Laboratorio de Biotecnología, Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Argentina

^e Departamento de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de La Rioja, Argentina

ARTICLE INFO

Article history:

Received 2 May 2016

Received in revised form 9 September 2016

Accepted 21 October 2016

Available online 24 October 2016

Chemical compounds studied in this article:

Ethylcellulose (PubChem CID: 24832091)

Keywords:

Ethylcellulose

Medium chain triglycerides

Surfactant

Chocolate rheology

β -V polymorph retention

XRPD

ABSTRACT

Chocolate manufacturing involves steps during which molten mass is prepared to be converted in a solid with selected textural and melting properties which are vital for the consumer's acceptance. A central element in this behavior is the continuous oily phase (cocoa butter), in which solid particles like sucrose and cocoa solids, refined to a selected particle size are dispersed and stabilized. Emulsifiers are important in particle coating and modify the viscosity of chocolate mass to give it adequate flowability during manufacturing. Furthermore, these molecules should not hinder the appearing of β -V polymorph during chocolate solidification.

In this work we evaluated ethylcellulose (EC) copolymers with different mean molecular weights (MMW) dissolved in medium chain triglycerides (MCT) oil, on the rheological and crystallization parameters of molten chocolate and cocoa butter. Rheology shows that EC induced a dose-dependent increase in yield value and viscosity, whereas MCT mainly induced a reduction in yield value. Therefore, by adjusting the EC concentration in the mixture it was possible to tune the rheological parameters of molten chocolate. Besides, X-ray diffraction showed that at the low proportions used to modify the rheological parameters (up to 0.2% EC and 1.9% TCM) EC-MCT blend did not affect the crystallization of cocoa butter which retained the β -V polymorph. The effects observed did not show a direct dependence with the MMW of EC suggesting that the modification of the rheological parameters is mostly thermodynamic (due to its surface activity) rather than kinetic (due to an increase in viscosity).

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Molten chocolate is a complex suspension of solid particles (sucrose and cocoa solids) dispersed into a fatty continuous phase of cocoa butter (CB) (Do, Hargreaves, Wolf, Hort, & Mitchell, 2007; Gloria & Sievert, 2001). Correct manufacturing of this suspension is vital to obtain a good quality solid product (Afoakwa, 2010a). The production process of chocolate can be summarized in four basic

stages: mixing, refining, conching and tempering (Afoakwa, 2010a). During conching, this dispersed system is thoroughly mixed until each particle is totally covered by CB, exhibiting a typical pseudoplastic behavior (Gonçalves & Da Silva Lannes, 2010; Jolly, Blackburn & Beckett, 2003). In order to achieve an efficient industrial manipulation of chocolate, different emulsifiers are often used as rheology regulators and to ensure coating of the solid particles (Schantz, Linke, & Rohm, 2003; Weyland & Hartel, 2010). Industrially, the flow behavior of chocolate is monitored through its apparent viscosity (AV) and yield value (YV), two rheological parameters which can be more or less individually handled through the use of emulsifier blends, generally lecithin and polyglycerol polyricinoleate (PGPR) (Afoakwa, Paterson, Fowler, & Vieira, 2009; ICA, 2000; Schantz & Rohm, 2005).

After conching, molten chocolate is turned into a solid through a carefully controlled thermal process, known as tempering (Sato,

Abbreviations: CB, cocoa butter; EC, ethylcellulose; MCT, medium chain triglycerides; MMW, mean molecular weight; MC, minor components; XRPD, x-ray powder diffraction.

* Corresponding author at: Centro de Excelencia en Productos y Procesos (CEPROCOR), Ministerio de Ciencia y Tecnología de Córdoba Pabellón CEPROCOR, CP 5164, Santa María de Punilla, Córdoba, Argentina.

E-mail addresses: ibianco@ceprocor.uncor.edu, ibianco@hotmail.com (I.D. Bianco).

2001). At this stage, the goal is to induce crystallization of CB into its β -V polymorph. Within the six possible CB polymorphic forms that can be identified by X-ray powder diffraction (XRPD), the β -V form confers the desirable textural properties and thermal resistance to chocolate (Izdiak, 2012; Talbot, 2009). In this regard, conching and tempering can be considered two indissoluble stages, therefore whatever is incorporated to aid in the conching step (i.e.: an emulsifier) may affect fat crystallization, impairing the proper development of the β -V form. The relevance of this aspect has been highlighted in previous works that showed that emulsifiers added as *minor components* could have important effects on the crystalline fat network (Garti & Sato, 1986; Garti, Wellner, & Sarig, 1981; Miskandar, Che Man, Abdul Rahman, Nor Aini, & Yusoff, 2007; Schlichter, Sarig, & Garti, 1987). In this regard, it is not defined from what concentration a substance must be considered as “minor”; usually that depends of both the proportion of the major components as well as the steps involved in the crystallization process. In this regard, it has been reported that minor components incorporated in the range of 0.5–5% and in certain cases below 0.1% may be enough to alter the crystalline structure (Smith, Bhaggan, Talbot, & Van Malsen, 2011). In CB, minor components can act by accelerating or retarding the transition from β -V to β -VI, phenomena that are usually referred as *β -V polymorph retention* (Dhonsi & Stapley, 2006; Garti, Schlichter, & Sarig, 1986; Schlichter, Sarig & Garti, 1985).

In a good quality chocolate, it is desired that β -V phase retention lasts as long as possible before its fusion and liquid fat migration towards chocolate surface, where triglycerides crystallize into the β -VI form. This unavoidable polymorphic transition, known as *fat bloom*, occurs in long term stored or bad tempered chocolates (Garti et al., 1986; Peschar et al., 2004), and is accelerated when using inappropriate emulsifiers. The evaluation of possible effects on β -V polymorph retention has been usually studied on CB, since the presence of crystalline sugar produces a diffraction pattern that overlaps and screens most of that coming from fat crystals. Even so, it has been shown that these studies in CB have an important predictive value for the occurrence of the fat bloom phenomenon in the final product (Marangoni et al., 2012; Peschar, et al., 2004; van Mechelen, Peschar, & Schenk, 2006). Nevertheless, few studies have simultaneously addressed the impact of emulsifiers on both the rheological behavior as well as on β -V polymorph retention. Furthermore, most of the reported studies only considered conventionally used emulsifiers like lecithin, PGPR and sorbitan esters, among others (Dhonsi & Stapley, 2006; Garti et al., 1986; Masuchi, Grimaldi, & Kieckbusch, 2014; Svanberg, Ahrné, Lorén, & Windhab, 2011). Since most of the lecithin that is produced comes from genetically modified (GM) soy and in some countries consumers express an important concern on the use of GM-derived ingredients in food products, there is a growing tendency in the search for new emulsifier alternatives. In this context, hydrophobic or amphiphilic polymers, which may act through entirely different mechanisms, offer an option that has been scarcely studied in foodstuffs (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; Ceballos et al., 2014; Do et al., 2010; Kralova & Sjöblom, 2009).

In order to evaluate the suitability of this strategy, our group started studies aimed at characterizing a novel emulsifier blend composed by the graft copolymer ethylcellulose (EC) dissolved into a mixture of medium chain triglycerides (MCT) in non-aqueous emulsions. MCT was chosen between different oils including CB, since the saturated fatty acids confer to MCTs great oxidative stability, very faint color, characteristic slight taste and turn it essentially odorless. Accordingly, the blends obtained with EC were essentially odorless, with very faint color and proper slight taste. But undoubtedly, its most remarkable feature was that MCT made it possible to avoid the overheating of CB to incorporate EC,

preserving its characteristic organoleptic properties. These studies also showed that the interfacial adsorption of EC on the surface of propylene glycol (PG) droplets plays a key role in the building-up and stabilization of PG-in-MCT emulsions (Ceballos et al., 2014). In agreement with Bancroft's rule, the high solubility of EC in oils allowed that MCT end up being the continuous phase of the system. This solubility is a consequence of the chemical structure of EC and represents one of the most important features of this copolymer related to its food applications, since it can be easily solubilized into vegetable oils and fats (Co & Marangoni, 2012; Gravelle, Barbut, & Marangoni, 2012; Stortz, Zetzl, Barbut, Cattaruzza, & Marangoni, 2012; Zetzl, Marangoni, & Barbut, 2012). This macromolecule has been designed through grafting of ethoxyl groups to the cellulose backbone. However, as most polymers, EC has been mainly used to increase the viscosity or even gel a solution (Co & Marangoni et al., 2012; Gravelle et al., 2012). In order to attain these effects a considerable amount of polymer has to be employed (usually $\geq 1\%$ by weight). In our previous work, we proved that the surface activity of this polymer has a stabilizing effect on non-aqueous emulsions independently of an alternative possible kinetic mechanism due to continuous phase thickening (Ceballos et al., 2014). According to these results and knowing that EC is soluble in CB, it is reasonable to expect that Bancroft's rule should be obeyed when the emulsifier blend (EC-MCT) is added to molten chocolate. Thus, it could be predicted that EC-MCT could have also an effect on both formation as well as stabilization of a proper chocolate suspension. In this context, it is expected that inclusion of a very small amount of the polymer into the CB crystalline network after tempering should not inhibit the arising of the β -V polymorph, neither should accelerate the transition from β -V to β -VI polymorph.

In this connection, research of Marangoni's group has demonstrated in chocolate models that EC can interact with sucrose and others particles (Stortz & Marangoni, 2013; Stortz, De Moura, Laredo, & Marangoni, 2014; Storts, Laredo, & Marangoni, 2015). Through several methods, these authors demonstrated that EC interacts with sucrose via hydrogen bonds and at concentrations around 2% favor the building-up of a network between different solid particles, resulting in a heat resistant chocolate. However, no mention was made respect to the flow behavior when these molten chocolates were conched. In our experience, at EC concentrations above 0.5% the molten chocolates become too viscous and its industrial manipulation is very hard.

Therefore, the purpose of the present study was to evaluate the emulsifying and rheology modifying properties of very low concentration solutions of EC in MCT when added to a complex food system like chocolate. The composition of this blend was modified in order to study its effects on both apparent viscosity and yield value when molten chocolate conching is carried out. In addition, the interfacial activity of EC was assessed as a possible mechanism of action of this anhydrous suspension on the rheological parameters of molten chocolate. Finally, the impact of EC-MCT on the β -V polymorph retention after CB tempering was explored by XRPD as a relevant and complementary aspect related to the polymeric surfactant activity.

2. Materials and methods

2.1. Materials

Dark chocolate samples without emulsifier addition were prepared in a pilot plant from Arcor División Chocolates (Colonia Caroya, Argentina). Its composition was as follows: 44.2% sucrose, 46.1% cocoa liquor, 9.7% CB. When emulsifiers were added, an equivalent amount of CB was removed to maintain these proportions. These samples were refined until a mean particle

size of 28 μm was achieved and then conched during 24 h at 60 °C, without tempering.

EC polymers of different mean molecular weights (MMW) were used: 77, 120, 158 and 220 kDa (product designation was Ethocel™ Standard Premium 10, 20, 45 and 100 respectively). The ethoxy content was between 48.0% and 49.5% (supplier information). These polymers were kindly provided by Dow Chemicals, Brazil.

Medium chain (caprylic/capric) triacylglycerol (MCT) oil (Neobee™ M-5) was obtained from Stepan Co (USA). This oil contains 98% of a mixture of C8:0 and C10:0 fatty acids (66% and 32% respectively).

2.2. Standard procedure for the preparation of EC solutions in oils

EC and MCT oil were weighed and mixed at room temperature, and then heated with constant stirring during 5 min on a hot plate (Thorbell; Argentina) set at 90 °C, in order to achieve a homogeneous suspension. Then, temperature was increased until the EC glass transition temperature (T_g) was reached (between 130 °C and 150 °C, depending on the MMW (mean molecular weight) of the polymer), which occurred within 10 min. According to our experience, all EC polymers in 50 g mixture batches form a clear solution in less than 5 min after the T_g is reached. After that, solutions were let to cool at room temperature until their use.

2.3. Addition of emulsifiers

Emulsifiers (lecithin and EC-TCM) at room temperature were gently added to chocolate samples previously melted at 55 °C. Afterwards, each sample was homogenized at the same temperature during 2 h using a blade mixer at 20 r.p.m. The EC-TCM blend was used at levels that allow to obtain chocolates with a similar rheological behavior when compared with a lecithin chocolate. In this way, lecithin was used at a commercial chocolate level (0.5%). All EC-TCM concentrations were calculated from the total weight of each sample, and are indicated in each corresponding figure caption.

2.4. Chocolate tempering

A manual tempering procedure was designed to obtain the chocolate β -V polymorph. Specifically, 100 g of solid chocolate into

a 250 mL beaker were warmed up at 55 °C in an oven (Shel Lab; USA) to ensure the complete melting of fat crystals. Afterwards and while removing, molten chocolate was cooled in a water bath at about 18 °C until a temperature of 29.3 °C was reached (cooling rate = 3 °C min^{-1}), in order to obtain a substantial number of stable β -V crystals. Finally, chocolate was heated in second water bath at about 30 °C until a temperature of 29.8 °C to melt the crystals of unstable polymorphic forms. Temperature control was carried out with Checktemp® digital thermometer (Hanna Instruments; USA). Immediately, molten chocolate was poured into a plastic mold and stored at 20 ± 2 °C at least 2 h before XRPD measurements.

2.5. Rheology measurements

Molten chocolate flow curves were performed by following the recommendations from the International Confectionery Association (ICA, 2000), at 40 °C in a controlled stress rheometer (Physica MCR 301; Anton-Paar; Austria) with a bob and cup geometry as measuring system. Briefly, measurements of shear stress and viscosity within 2 s^{-1} and 50 s^{-1} of shear rates were carried out, using up and down curves in shear rate, preceded by a pre-shear at 5 s^{-1} during 5 min.

As referred in several reports, according to ICA recommendations, YV and viscosity results can be expressed as shear stress and viscosity respectively, and are obtained at defined shear rates from the ramp up in the flow curve; or by fitting the flow curve with a mathematical model and extracting the resultant regression parameters (Afoakwa et al., 2009; Afoakwa, 2010b; Beckett, 2009; Do et al., 2010; Do, Vieira, Hargreaves, Wolf, & Mitchell, 2008). Following the recommendations of Servais, Ranc, and Roberts (2004) and multiple referenced work (Afoakwa et al., 2009; Afoakwa, 2010b; Do et al., 2010; Gonçalves & Da Silva Lannes, 2010), in this work we report the value of stress at a shear rate of 5 s^{-1} as the YV, and the value of viscosity at 40 s^{-1} as representing the apparent viscosity. From an industrial viewpoint, these authors consider this recommendation as a practical way whereby both parameters can be measured, also providing a clear physical significance.

When necessary, rheograms will be presented as either fitting average curve, especially for better visualization, as well as the total analysis curve (up and down ramps) will be showed to explain hysteresis phenomena of chocolate samples.

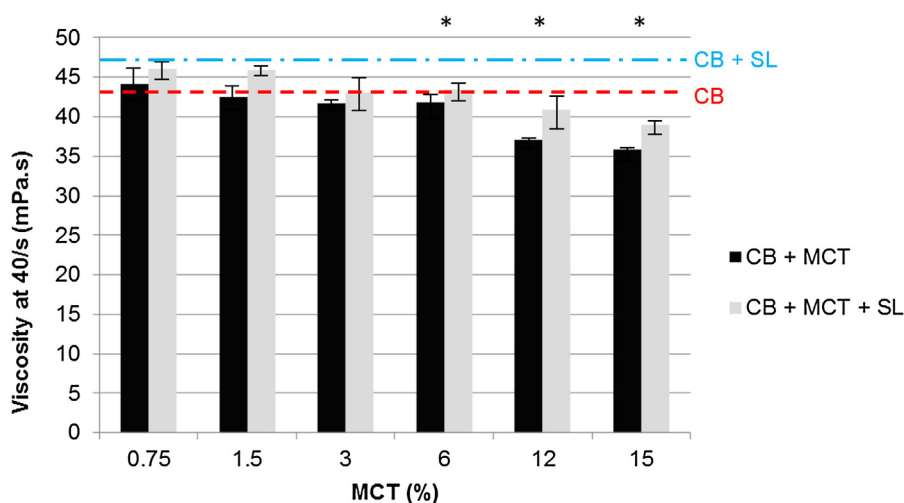


Fig. 1. Effect of MCT in different concentrations on viscosity at $\text{SR} = 40/\text{s}$ for CB (dotted red line) and CB + 0.5% soy lecithin (SL) blends (dotted blue line). Error bars are SD. Asterisks indicate significant differences ($p < 0.05$).

2.6. XRPD measurements

X ray diffraction data collection was performed at 25 °C in a D8 Advance X-ray powder diffractometer (Bruker; USA). XRD patterns were recorded from 2° to 40° 2θ (CuKα; λ = 1,5418 Å, 40 kV, 40 mA). Data processing was performed through DIFFRACplus EVA software.

2.7. Sample preparation for XRPD measurements

After tempering and kept at 20 ± 2 °C for at least 2 h, chocolate samples were demolded and grated with a kitchen device. This sample sizing standardization was performed to prevent preferred orientation phenomenon. After size reduction, samples were put into the sample holder avoiding its compaction.

2.8. Statistical analysis

Results are shown as mean values ± standard deviation (SD) of three measurements. Analysis of variance (ANOVA) was used to determine if significant differences existed between samples for the parameters YV and apparent viscosity at 95% confidence level. Experimental results were analyzed using Infostat software (Facultad de Agronomía, Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

3.1. Chocolate flow behavior and emulsifier performance

As mentioned above, the use of emulsifier mixtures to modify rheological parameters of chocolate mass is a very common practice in chocolate manufacturing. In this connection, we found recently that EC dissolved in MCT stabilizes non-aqueous emulsions mostly due to its interfacial properties rather than by its thickening effect (Ceballos et al., 2014). Even though MCT was used for the dissolution of EC and thus to facilitate its incorporation to chocolate mass, we found that the incorporation of MCT alone affects the flow properties of CB (Fig. 1) CB/sucrose blends (Fig. 2a and b) and chocolate (Fig. 3a and b). Due to its chemical composition, MCT is liquid at room temperature. Therefore, as reasonably expected, a fluidizing effect occurred when it was added to CB or its former mentioned blends. As rheological measurements were carried out at 40 °C (see Materials and methods) CB is melted and showed practically Newtonian behavior with a dose dependent fluidizing effect of MCT (Fig. 1). In this regard, that phenomenon was also observed even in the presence of lecithin previously incorporated to CB (Fig. 1).

Nevertheless, in order to be useful for chocolate manufacturing, the emulsifying effect should be evidenced in the presence of sucrose, reducing the friction between the suspended particles and facilitating chocolate flow (Windhab, 2006). Interestingly, as shown in Fig. 2a, the fluidizing effect of MCT was observed even in the presence of the relatively high proportion of sucrose (around 45%) that is currently used in chocolate and contributes to its high viscosity.

It is important to note that, contrarily to what is observed on pure CB, lecithin induced an increase in YV as well as a decrease in viscosity at 40 s⁻¹ on a mixture of CB with sucrose Fig. 2a. This reduction was even to lower viscosities than that attained upon addition of a similar proportion of MCT. Interestingly, the addition of MCT containing a small amount of EC induced changes in the rheogram of CB/sucrose that resemble those attained upon addition of lecithin (increase in YV and decrease in viscosity at 40 s⁻¹ (Fig. 2a).

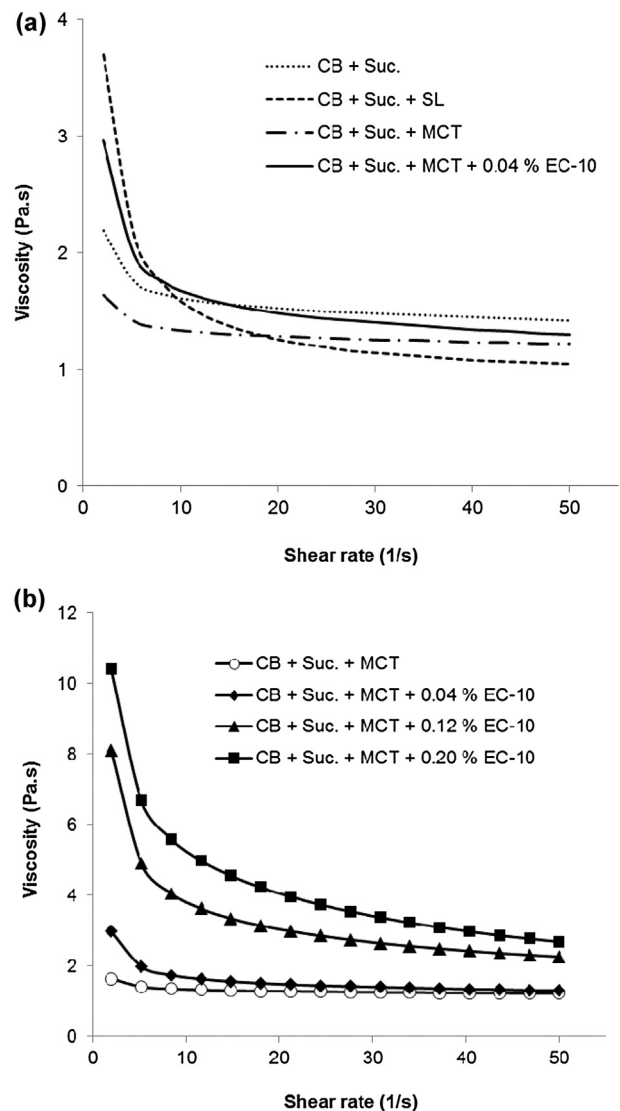


Fig. 2. Viscosity as a function of shear rate for: (a) A sucrose-in-CB suspension and its blends with MCT and MCT+EC-10. For comparison, a sample of sucrose-in-CB suspension with 0.5% of soy lecithin (SL) was included. (b) sucrose-in-CB suspensions with addition of different proportions of EC-10 dissolved in MCT: 0.20% EC-10 (filled square), 0.12% EC-10 (filled triangle), 0.04% EC-10 (filled diamond). For comparison, a sample of sucrose-in-CB suspension with only MCT was included (open circle). Composition of sucrose-in-CB suspension: 44.2% sucrose, 55.8% CB. When MCT or EC-MCT were added, an equivalent amount of CB was removed to maintain the proportions. The content of MCT in all samples was 1.0%. SD bars were smaller than the size of the symbols used. YV and viscosity at SR = 40/s were statistically different ($p < 0.05$), except for viscosity at SR = 40/s for (b) CB + Suc. + MCT + 0.04% EC-10 ($p > 0.05$).

As expected, a dose-dependent increase in YV and viscosity was observed upon increasing EC concentration (Fig. 2b). However, this effect was more pronounced on YV rather than on viscosity at 40 s⁻¹.

EC produced a dose-dependent structuring effect, and the suspensions of sucrose in CB showed an increasingly marked pseudoplastic behavior (Fig. 2b). In this context, as EC is increased it could even have a gelling effect on MCT which could also be propagated to CB (Sortz & Marangoni, 2014). In agreement with these findings, when MCT was added to a chocolate prepared without emulsifiers, a marked dose-dependent fluidizing effect was observed (Fig. 3a), with significant reductions in YV and viscosity at 40 s⁻¹ (Fig. 3b).

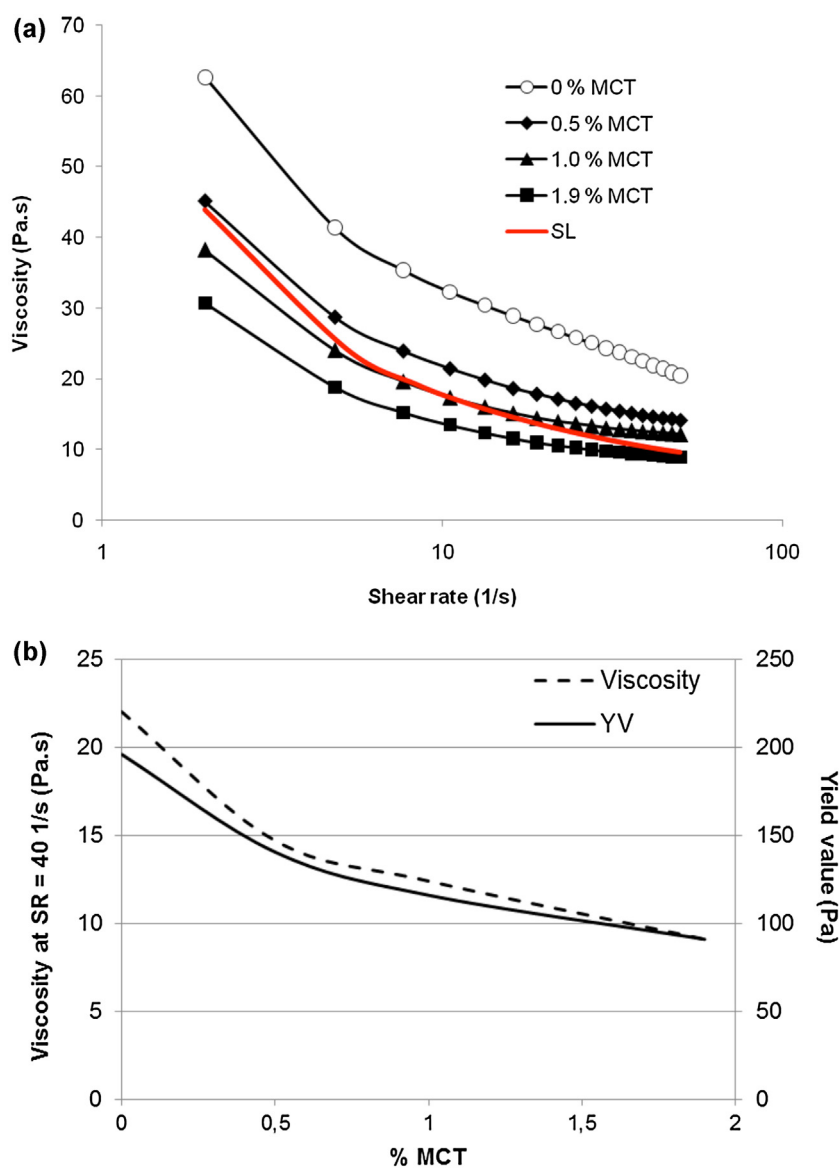


Fig. 3. Impact of the MCT content on the dark chocolate flow behavior and its comparison with the effect of soy lecithin (SL; red filled line) over all range of shear rate measurements (a) and specifically on viscosity at SR = 40/s and YV (b). Composition of chocolate without emulsifiers: 44.2% sucrose, 46.1% cocoa liquor, 9.7% CB. When MCT was added, an equivalent amount of CB was removed to maintain the proportions. SD bars were smaller than the size of the symbols used.

When compared with soy lecithin (red line in Fig. 3a) MCT decreases both viscosity as well as YV in chocolate with an effect that is proportionally higher on YV.

In order to gain insight into the molecular mechanisms underlying EC effects on the rheological parameters of chocolate, we evaluated several EC-MCT blends. Testing was carried out at different polymer concentrations and using ECs with different MMW, keeping constant the final proportion of MCT in the mixture at 1% (w/w). As previously shown on a simpler suspension of sucrose particles in CB, YV and viscosity of chocolate mass increased as a function of EC concentration (Fig. 2b). Unexpectedly, the effect of EC on the rheological parameters of chocolate was anything but straightforward (Fig. 4). At very low concentrations of EC (i.e. between 0.04–0.07%), YV and viscosity surprisingly showed a decrease for the three EC with higher MMW as compared with the results obtained with the EC with the lowest MMW. Viscosity values ranged around those corresponding to the lecithin effect; whereas practically all YV were below those obtained with the phospholipid (Fig. 4).

Altogether, these results suggest that the effects of MCT-EC on the stabilization of chocolate are not due to an increase in viscosity caused by the incorporation of the polymer and are rather due to its interaction with solid particles of chocolate which is in agreement with previous reports using relatively high concentrations of EC (Stortz & Marangoni, 2014).

The very low concentrations at which EC modifies the rheological parameters of chocolate represent an advantage with respect to the classical industrial emulsifiers, since it will require the preparation of low viscosity solutions of EC.

As a reference, 0.10% is the concentration that, at least for the three EC with higher MMW, clearly produced a sharper increase on both parameters, but where the YV increment is proportionally higher. This effect allows predicting that from this concentration, EC can be used to raise the YV in some types of chocolate (e.g. chocolate coatings).

On the other hand, as can be seen in Fig. 4, MCT-EC mixtures with a higher proportion of MCT decreased YV, which can be useful for bar chocolates for faster fluidization into hot milk. Clearly, these

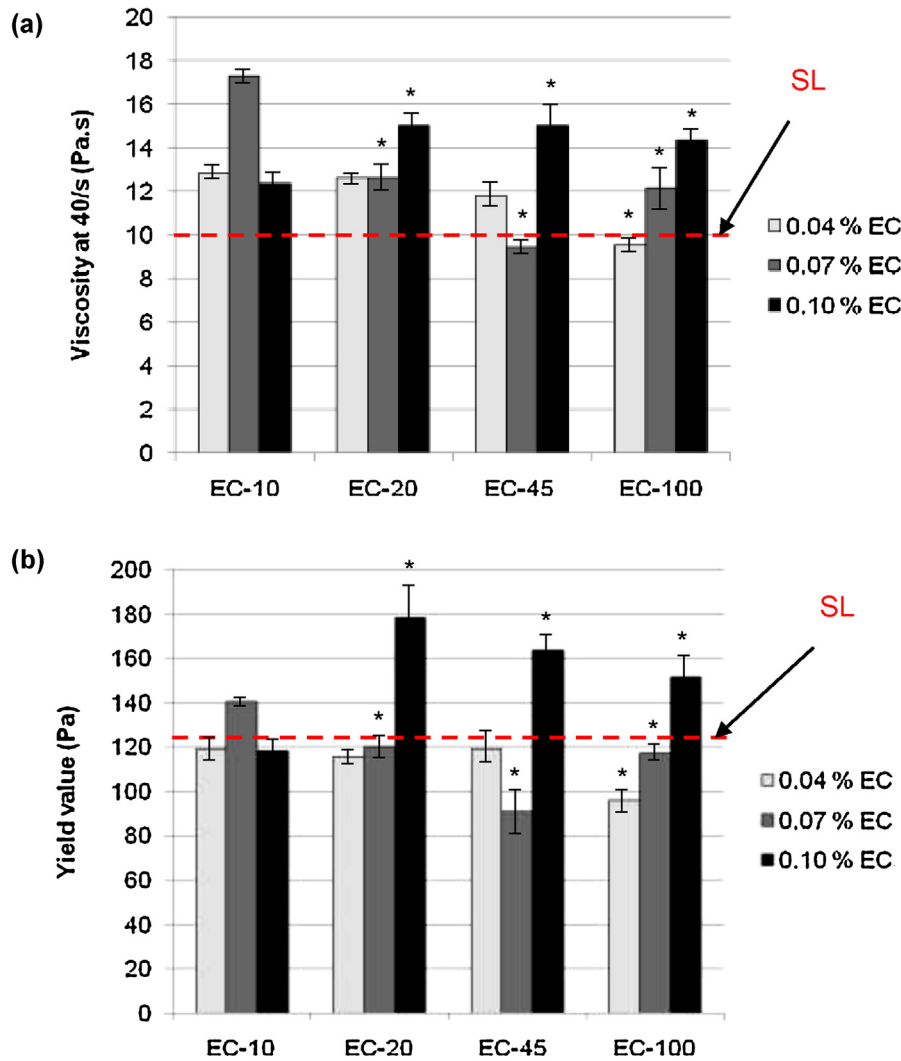


Fig. 4. Effect of the content and MMW of EC on viscosity at SR = 40/s (a) and YV (b) of dark chocolate. For comparison, values of these parameters for a chocolate with 0.5% soy lecithin (SL) were included (red dotted line). Composition of chocolate without emulsifiers: 44.2% sucrose, 46.1% cocoa liquor, 9.7% CB. When EC-MCT was added, an equivalent amount of CB was removed to maintain the proportions. The concentrations of EC-MCT solutions were such that the content of MCT in all samples was 1.0%. Error bars are SD. At each concentration, bars marked with an asterisk are statistically different than EC-10 at the same concentration ($p < 0.05$).

effects on YV are related to changes in viscosity, which will affect mostly the energy involved in the conching step during the industrial processing of chocolate (Bolenz, Thiessenhusen, & Schäpe, 2003; Jolly et al., 2003). In this context, at concentrations of EC above 0.10% by weight, the mass of chocolate becomes too plastic and more work is required to mix it after emulsifier addition. It has been recently shown that at concentrations above 2% by weight EC can be used to prepare heat resistant chocolate (Sortz & Marangoni, 2013). Further studies demonstrated that EC forms hydrogen bonds with sucrose therefore forming a network that by trapping the oil phase leading to structuring and heat resistance (Stortz et al., 2014). These results provide the basis for the increase in YV that is observed upon addition of EC to chocolate. The results reported herein also indicate that this interaction also aids in the conching process of chocolate that has the purpose to cover solid particles with fat. As mentioned above, molten chocolate behaves as a non-Newtonian pseudoplastic fluid which shows a shear thinning behavior when the YV has been exceeded (Fig. 3a). This phenomenon is clearly time-dependent, and develops when rising shearing forces are applied during chocolate mass conching. This increase in shear stress induces the

collapsing of the semi-solid tridimensional structure of chocolate, and the asymmetric shaped particles which have been released of that broken network begin to align within the resulting stream. As a consequence, viscosity decreases and becomes virtually independent of shearing at high shear rates (Windhab, 2006).

In addition, after refining a high proportion of those asymmetric particles tend to clump together. For this reason, conching works as an agglomeration breakdown process, since flowing and mixing of the chocolate suspension allows covering of each individual particle with fat (CB) with the consequent breaking of lumps. Thus, if an emulsifier is efficient, that phenomenon will be favored, and a more creamy chocolate will be obtained. Moreover, reducing viscosity of the mass by the use of emulsifiers will be translated to reduced operating costs not only by a reduction in the energy required for the conching process but also because less amount of the most expensive raw materials (i.e.: CB) are required to attain a high quality product.

The area between up and down ramps of whole rheograms implies a hysteresis phenomenon whose magnitude will be indicative of the conching efficiency as well as the emulsifying performance of the ingredients in the fat phase. The time-

dependence of this phenomenon is indicative of thixotropy which is also related to the efficiency of the emulsifiers during conching. Consequently, a well conched chocolate is not thixotropic. It was observed that chocolate samples containing only MCT displayed thixotropy (Fig. 5, blue curve). On the other hand, the addition of EC improved the emulsifying efficiency of MCT reducing the hysteresis of the rheograms indicating that its mixture with MCT has a performance equivalent to that of a good emulsifier (Fig. 5, red curve).

Although MCT could be used to reduce chocolate mass viscosity it also reduces YV. We found that the incorporation of a very small amount of the linear polymer EC allows increasing YV while retaining the benefits of a low viscosity of the chocolate mass at high shear rates. Previous studies using EC described a surfactant action of this polymer on w/o emulsions (Hasenhuettl, 2010; Melzer, Kreuter, & Daniels, 2003). Considering both chocolate anhydrous nature as well as its inverse suspension structure (polar-in-apolar dispersion) we designed a water-free model system to explore on its molecular mechanism. In that study, using propylene glycol-in-oil emulsions we demonstrated that EC stabilizes and lowers the energy for the formation of these non-aqueous emulsions through an interfacial stabilization mechanism rather than a kinetic action through a thickening of the system (Ceballos et al., 2014). These results are in agreement with those found by Melzer et al. (2003). The results reported herein show that the effects of EC in chocolate mass rheology are not dependent on its MMW and have been observed at very low concentrations (within 0.04 and 0.07% w/w) both suggesting also an interfacial stabilization mechanism due to its interaction with solid particles present in chocolate.

As previously mentioned, stabilization/destabilization phenomena of emulsions and suspensions have been invariably described through water-dependent mechanisms. Nevertheless, these events can be applied in an attempt to explain the observations on anhydrous systems like non-aqueous emulsions and chocolate.

It has been well established that graft polymeric surfactants behave in such a way that their hydrophobic portions interact mainly with the hydrophobic phase of an emulsion while their hydrophilic portions are disposed interacting mainly with the hydrophilic phase (Tadros, 2009).

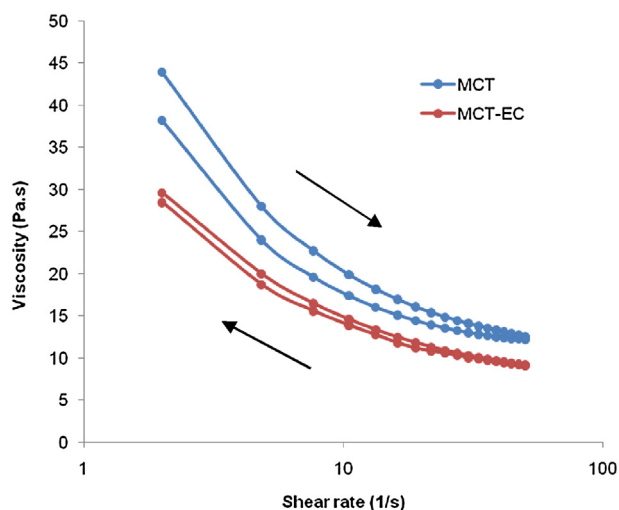


Fig. 5. Viscosity as a function of shear rate for dark chocolate samples with 1% MCT (blue curve) and 1.07% MCT – EC-45 blend (1% MCT – 0.07% EC-45, red curve). Arrows indicate data obtained when the increasing and decreasing shear rate ramps were recorded.

In dark chocolate, conjunction of water absence and the high hydrophobicity of EC limit the options of polymer solubility. However, considering that the more polar component in chocolate suspension is the surface of the solid components incorporated into CB (sucrose and cocoa solids) and the more polar component in the fat phase is the backbone of EC, it is reasonable to assume that during conching EC end covering the surface of sucrose. Thereby, grafted ethoxyl groups will mostly interact with triglycerides in CB and the more polar central polymeric chain end interacting mostly with the surface of sucrose, causing thus the necessary free energy reduction that avoids destabilization and further phase separation of the suspension (see Graphical Abstract). In this context, it has been recently demonstrated using molecular dynamics and FTIR that EC forms hydrogen bonds with sucrose (Stortz & Marangoni, 2014). As a consequence, at concentrations above 2% by weight the addition of EC to chocolate leads to structure formation which was used for the preparation of heat resistant chocolate (Stortz & Marangoni, 2014). In this work we have shown that at low concentrations the interactions between EC and sucrose avoid their interaction and lump formation favoring chocolate conching (Fig. 5).

Nonetheless, if a certain critical value of EC concentration is exceeded, it will produce an excess of polymer molecules in the continuous phase which leads to structure formation that has been the basis for its use in the preparation of heat resistant chocolate (Stortz & Marangoni, 2014).

3.2. Effect of EC-MCT on CB crystallization

Consumer's acceptance of chocolate and other crystalline fat-based foodstuffs (e.g. butter, margarine) is conditioned by the plastic properties of its constituent fats. Fusion and crystallization are the most important features and both are intimately related to its composition of TG. The structure of this continuous phase provides the outstanding organoleptic characteristics in fats, making that foods can be perceived as creamy rather than brittle; or soft rather than sandy (Smith et al., 2011). In this connection, it is well known that foreign materials, although added as minor components, can affect fat crystallization either at the macroscopic or microscopic level. Microscopically, fat crystallization could be influenced at different stages namely nucleation, crystal growth and morphology, heat capacity or polymorphic stability. Related to CB and chocolate, any of these stages being affected would be macroscopically translated into fat bloom development, besides melting profile alteration, over-hardening and defective rheological behavior (Smith et al., 2011).

In this context, as the presence of foreign materials like a polymeric emulsifier as EC dissolved in liquid oil might influence the crystallization properties of CB it was relevant to evaluate its effect on the final polymorphic state of the fat.

As mentioned above, the selection of an emulsifier for molten chocolate is based not only on its good performance as rheological regulator. The selected surfactant should not avoid the arising of β -V polymorph as the more stable crystalline form of CB when chocolate undergoes liquid-solid transition. Additionally, it could also retard the arising of β -VI polymorph, which is indicative of fat bloom deterioration. Combination of these events is referred as the capacity of β -V polymorph retention (Dhonsi & Stapley, 2006; Garti et al., 1986; Schlichter et al., 1985).

Although there are studies about the influence on fat crystallization of different emulsifiers usually employed in chocolate manufacturing, the underlying mechanisms of polymeric surfactants on this process have not been thoroughly studied.

A possible perturbation on the crystalline fat network would be present when considering molecular size difference between EC and lecithin phospholipids. In addition, when ECs with different

MMW (see Materials and Methods) are taken into account, that size disturbing action could be significantly different too. In any case, the possible perturbing effect on CB network could be due to an event of steric nature, therefore related to the MMW of the polymer.

DRX patterns for CB and its blends with the different ECs studied are shown in Fig. 6. As can be seen, CB crystallized into β -V polymorph even at the highest concentrations and MMW of EC that were successfully used as rheology modifiers in molten chocolate.

4. Conclusions

The results reported herein show that a solution of EC in MCT can be suitably used as rheology modifier in chocolate manufacturing. The main features of this mixture are its effortless preparation, simple dosage and easiness to adjust the rheological parameters in order to produce diverse types of dark chocolate. In this sense, viscosity and yield value in molten chocolate can be modified

according to specific requirements. Related to the solid final product, we found that the addition of EC-MCT does not interfere with the crystallization of CB which after a standard tempering develops its β -V polymorph leading to stable chocolates. The absence of a direct dependence with the MMW of EC suggests that the modification of the rheological parameters is mostly thermodynamic (due to its surface activity) rather than kinetic (due to an increase in viscosity).

Acknowledgments

This work was supported by grants from CEPROCOR and CONICET to Drs. Beltramo and Bianco, PIP 11220100100502 and PIP 11220090100732. The authors declare no conflict of interest. K.B., DMB and IDB are research staff of CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas).

The authors thank Florence Amaral from Dow Wolff Cellulosics, São Paulo, Brazil, for providing the Ethocel samples. Thanks are extended to Dr. Miriam Strumia, Departamento de Química

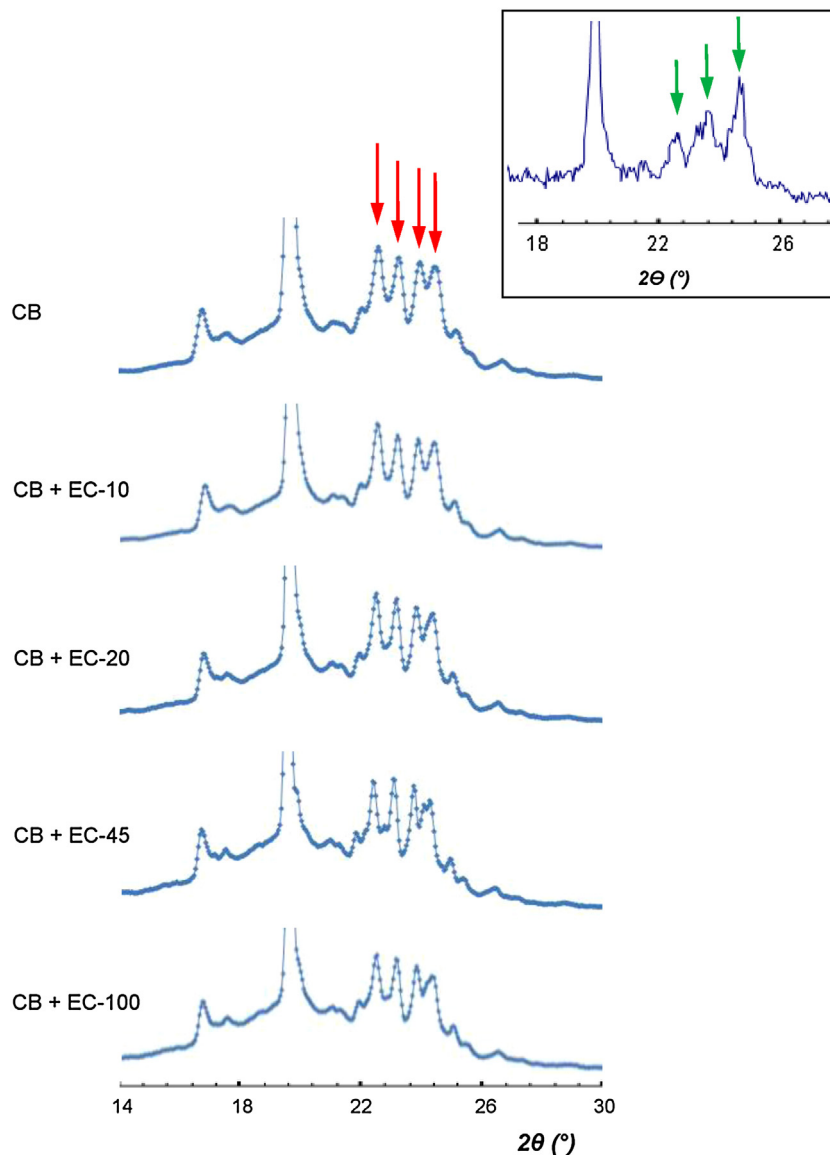


Fig. 6. X-ray diffraction powder patterns of the fingerprint region of form β -V in cocoa butter and its blends with the MCT (1% w/w)-EC (0.08% w/w) emulsifier mixture. Red arrows indicate the characteristic reflections of β -V polymorph. Rectangle: XRPD pattern of fingerprint region of form β -VI in pure cocoa butter, with green arrows indicating its characteristic reflections (for comparison only).

Orgánica, Universidad Nacional de Córdoba, Argentina, for permission to use the rheometer.

The authors are indebted to the members of Arcor R & D Division, W. Stark, L. A. Ceballos, D. Argüello and F. Rencini for providing the chocolate samples without emulsifiers and for their support and continuous interest in our work.

Special thanks to Ignacio Silva from CEPROCOR, for his support in the Graphical Abstract design.

References

- Afoakwa, E. O., Paterson, A., Fowler, M., & Vieira, J. (2009). Comparison of rheological models for determining dark chocolate viscosity. *International Journal of Food Science and Technology*, *44*, 162–167.
- Afoakwa, E. O. (2010a). Industrial chocolate manufacture – processes and factors influencing quality. In E. O. Afoakwa (Ed.), *Chocolate science and technology* (pp. 35–57). United Kingdom: Wiley-Blackwell.
- Afoakwa, E. O. (2010b). Structure – properties (rheology, texture and melting) relationships in chocolate manufacture. In E. O. Afoakwa (Ed.), *Chocolate science and technology* (pp. 101–154). United Kingdom: Wiley-Blackwell.
- Beckett, S. T. (2009). Chocolate flow properties. In S. T. Beckett (Ed.), *Industrial chocolate manufacture and use* (pp. 224–246). United Kingdom: Wiley-Blackwell.
- Bolenz, S., Thiessenhusen, T., & Schäpe, R. (2003). Fast conching for milk chocolate. *European Food Research and Technology*, *218*, 62–67.
- Bouyer, E., Mekhloufi, G., Rosilio, V., Grossiord, J. L., & Agnely, F. (2012). Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field? *International Journal of Pharmaceutics*, *436*(1–2), 359–378.
- Ceballos, M. R., Brailovsky, V., Bierbrauer, K. L., Cuffini, S. L., Beltramo, D. M., & Bianco, I. D. (2014). Effect of ethylcellulose on the structure and stability of non-aqueous oil based propylene glycol emulsions. *Food Research International*, *62*, 416–423.
- Co, E. D., & Marangoni, A. G. (2012). Organogels: An alternative edible oil-structuring method. *Journal of the American Oil Chemists' Society*, *89*, 749–780.
- Dhonsi, D., & Stapley, A. G. F. (2006). The effect of shear rate, temperature: Sugar and emulsifier on the tempering of cocoa butter. *Journal of Food Engineering*, *77*, 936–942.
- Do, T.-A. L., Mitchell, J. R., Wolf, B., & Vieira, J. (2010). Use of ethylcellulose polymers as stabilizer in fat-based food suspensions examined on the example of model reduced-fat chocolate. *Reactive & Functional Polymers*, *70*, 856–862.
- Do, T.-A. L., Hargreaves, J. M., Wolf, B., Hort, J., & Mitchell, J. R. (2007). Impact of particle size distribution on rheological and textural properties of chocolate models with reduced fat content. *Journal of Food Science E: Food Engineering and Physical Properties*, *72*(9), E541–E552.
- Do, T.-A. L., Vieira, J., Hargreaves, J. M., Wolf, B., & Mitchell, J. R. (2008). Impact of limonene on the physical properties of reduced fat chocolate. *Journal of the American Oil Chemists' Society*, *85*, 911–920.
- Garti, N., & Sato, K. (1986). Effects of surfactants on transition kinetics of stearic acid polymorphs. *Journal of the American Oil Chemists' Society*, *63*(2), 236–239.
- Garti, N., Schlichter, J., & Sarig, S. (1986). Effect of food emulsifiers on polymorphic transitions of cocoa butter. *Journal of the American Oil Chemists' Society*, *63*(2), 230–236.
- Garti, N., Wellner, E., & Sarig, S. (1981). Effect of food emulsifiers on crystal structure and habit of stearic acid. *Journal of the American Oil Chemists' Society*, *58*, 1058–1060.
- Gloria, H., & Sievert, D. (2001). Changes in the physical state of sucrose during dark chocolate processing. *Journal of Agricultural and Food Chemistry*, *49*, 2433–2436.
- Gonçalves, E. V., & Da Silva Lannes, S. C. (2010). Chocolate rheology. *Ciência E Tecnologia De Alimentos*, *30*(4), 845–851.
- Gravelle, A. J., Barbut, S., & Marangoni, A. G. (2012). Ethylcellulose oleogels: Manufacturing considerations and effects of oil oxidation. *Food Research International*, *48*, 578–583.
- Hasenhuettl, G. (2010). Synthesis and commercial preparation of food emulsifiers. In G. L. Hasenhuettl, & R. W. Hartel (Eds.), *Food emulsifiers and their applications* (pp. 11–37). 2nd ed. New York: Springer.
- International Confectionery Association (ICA). (2000) Viscosity of cocoa and chocolate products. Analytical method 46. Bruxelles, Belgium.
- Izdiak, S. H. J. (2012). Powder x-ray diffraction of triglycerides in the study of polymorphism. In A. G. Marangoni (Ed.), *Structure-function analysis of edible fats* (pp. 79–106). Urbana: AOCS Press.
- Jolly, M. S., Blackburn, S., & Beckett, S. T. (2003). Energy reduction during chocolate conching using a reciprocating multihole extruder. *Journal of Food Engineering*, *59*, 137–142.
- Kralova, I., & Sjöblom, J. (2009). Surfactants used in food industry: A review. *Journal of Dispersion Science and Technology*, *30*, 1363–1383.
- Marangoni, A. G., Acevedo, N., Maleky, F., Co, E., Peyronel, F., Mazzanti, G., et al. (2012). Structure and functionality of edible fats. *Soft Matter*, *8*, 1275–1300.
- Masuchi, M. H., Grimaldi, R., & Kieckbusch, T. G. (2014). Effects of sorbitan monostearate and monooleate on the crystallization and consistency behaviors of cocoa butter. *Journal of the American Oil Chemists' Society*, *91*, 1111–1120.
- Melzer, E., Kreuter, J., & Daniels, R. (2003). Ethylcellulose: A new type of emulsion stabilizer. *European Journal of Pharmaceutics and Biopharmaceutics*, *56*, 23–27.
- Miskandar, M. S., Che Man, Y. B., Abdul Rahman, R., Nor Aini, I., & Yusoff, M. S. A. (2007). Effects of emulsifiers on crystal behavior of palm oil blends on slow crystallization. *Journal of Food Lipids*, *14*, 1–18.
- Peschar, R., Pop, M. M., De Ridder, D. J. A., van Mechelen, J. B., Driessen, R. A. J., & Schenk, H. (2004). Crystal structures of 1, 3-distearoyl-2-oleoylglycerol and cocoa butter in the $\beta(V)$ phase reveal the driving force behind the occurrence of fat bloom on chocolate. *Journal of Physical Chemistry B*, *108*, 15450–15453.
- Sato, K. (2001). Crystallization behaviour of fats and lipids – A review. *Chemical Engineering Science*, *56*, 2255–2265.
- Schantz, B., Linke, L., & Rohm, H. (2003). Effect of different emulsifiers on rheological and physical properties of chocolate. *Proceedings of the 3rd international symposium on food rheology and structure* (pp. 329–333).
- Schantz, B., & Rohm, H. (2005). Influence of lecithin-PCPR blends on the rheological properties of chocolate. *LWT-Food Science and Technology*, *38*, 41–45.
- Schlichter, J., Sarig, S., & Garti, N. (1987). Mechanistic considerations of polymorphic transformations of triestearin in the presence of emulsifiers. *Journal of the American Oil Chemists' Society*, *64*(4), 529–533.
- Schlichter, J., Sarig, S., & Garti, N. (1985). Polymorphic transformations of cocoa butter in the presence of emulsifier studied by the DSC. *Thermochimica Acta*, *85*, 517–520.
- Servais, C., Ranc, H., & Roberts, I. D. (2004). Determination of chocolate viscosity. *Journal of Texture Studies*, *34*, 467–497.
- Smith, K. W., Bhagga, K., Talbot, G., & Van Malssen, K. F. (2011). Crystallization of fats: Influence of minor components and additives. *Journal of the American Oil Chemists' Society*, *88*, 1085–1101.
- Stortz, T. A., Zetzl, A. K., Barbut, S., Cattaruzza, A., & Marangoni, A. G. (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technology*, *24*(7), 151–154.
- Stortz, T. A., & Marangoni, A. G. (2013). Ethylcellulose solvent substitution method for preparing heat resistant chocolate. *Food Research International*, *51*, 797–803.
- Stortz, T. A., De Moura, D. C., Laredo, T., & Marangoni, A. G. (2014). Molecular interactions of ethylcellulose with sucrose particles. *RSC Advances*, *4*, 55048–55061.
- Storts, T. A., Laredo, T., & Marangoni, A. G. (2015). The role of lecithin and solvent addition in ethylcellulose-stabilized heat resistant chocolate. *Food Biophysics*, *10*, 253–263.
- Svanberg, L., Ahrné, L., Lorén, N., & Windhab, E. (2011). Effect of sugar: Cocoa particles and lecithin on cocoa butter crystallisation in seeded and non-seeded chocolate model systems. *Journal of Food Engineering*, *104*, 70–80.
- Tadros, T. (2009). Polymeric surfactants in disperse systems. *Advances in Colloid and Interface Science*, *147–148*, 281–299.
- Talbot, G. (2009). Chocolate temper. In S. T. Beckett (Ed.), *Industrial chocolate manufacture and use* (pp. 261–275). 4th ed. United Kingdom: Blackwell Publishing.
- van Mechelen, J. B., Peschar, R., & Schenk, H. (2006). Structures of mono-unsaturated triacylglycerols. II. The β_2 polymorph. *Acta Crystallographica Section B*, *B62*1131–1138.
- Weyland, M., & Hartel, R. (2010). Emulsifiers in confectionery. In G. L. Hasenhuettl, & R. W. Hartel (Eds.), *Food emulsifiers and their applications* (pp. 285–305). 2nd ed. New York: Springer.
- Windhab, E. (2006). What makes for smooth, creamy chocolate. *Physics Today*, *59*(6), 82–83.
- Zetzl, A. K., Marangoni, A. G., & Barbut, S. (2012). Mechanical properties of ethylcellulose oleogels and their potential for saturated fat reduction in frankfurters. *Food & Function*, *3*, 327–337.