Experimental report

11/09/2025

Proposal: 9-10-1747 **Council:** 10/2022

Title: Determination of the self-assemblyof minor lipidic components added to chocolate controlling the tempering process

Research area: Other...

This proposal is a new proposal

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Samples: Phospholipid

Instrument	Requested days	Allocated days	From	To
D22	2	2	17/06/2023	19/06/2023
D33	2	0		

Abstract:

Chocolate manufacturing is not easy and requires a well-controlled tempering (mixing and
heating) procedure to direct the crystallization of cocoa butter towards the formation of optimal cocoa butter crystals to obtain the texture, grain size, smoothness and shininess of chocolate. Our group has recently discovered that the addition of small lipidic components (phosphatidylcholine and phosphatidylethanolamine) promoted proper chocolate tempering without complex tempering procedures. This simpler tempering process would save time, money and energy, while decreasing waste, for big manufacturers. Now, our aim is to determine the mechanism of action of these minor lipids at the nanoscale. SANS is the most suitable technique to characterize the self-assembly of these minor lipidic components in cocoa butter and in commercial chocolate. Commercial deuterated phospholipids will be used.

Proposal number: 9-10-1747 on **D22** (17-18/06/2023)
Local Contact: PREVOST Sylvain
Participants : FAMEAU Anne-Laure, ARELLANO Helena
Proposal title: ***Determination of the self-assembly of minor lipidic components added to chocolate controlling the tempering process***

1/Background

Chocolate has played an important role in human culture since about 350 BC, when the Aztecs were drinking fermented cocoa. Today, chocolate gives psychological pleasure all around the world. Chocolate manufacturing is not easy and requires a well-controlled tempering (mixing and heating) procedure to direct the crystallization of cocoa butter to optimize the texture, grain size, smoothness and shininess of chocolate.[1] Our group has recently discovered that the addition of small lipidic components (phosphatidylcholine or phosphatidylethanolamine) to refined cocoa butter and chocolate promoted proper chocolate tempering without complex tempering procedures.[2] The addition of phosphatidylcholine or phosphatidylethanolamine at low concentration followed by cooling to 20 °C in the absence of shear, accelerates crystallization, stabilizes the desirable Form V polymorph of cocoa butter and induces the formation of chocolate with an optimal microstructure, surface gloss and mechanical strength. Final chocolate structure and properties are comparable to those of a commercial tempered chocolate. Minor lipidic component addition represents an effective way to engineer chocolate material properties at different length scales, thus simplifying the entire tempering process. This simpler, and more sustainable, tempering process, without the use of complex temperature and mixing protocols would save time, money and energy, while decreasing waste, for big manufacturers, and would also open possibilities for smaller manufacturers to produce high quality chocolate. Now, our aim was to determine the mechanism of action of these minor lipids at the nanoscale. SANS was the most suitable technique to characterize the self-assembly of these minor lipidic components in cocoa butter and in commercial chocolate.
In our previous study, we showed that phosphatidylcholine or phosphatidylethanolamine can be employed as effective additives for engineering the crystallization behavior, polymorphism, nanostructure and microstructure in cocoa butter and chocolate products. [2] The mechanism of action of these phospholipids introduced in the hot molten phase of cocoa butter remained however unknown. The aim of this proposal was to determine the structure of these phospholipids in the hot molten phase to understand how they can control the crystallization behavior and polymorphism leading to the adequate microstructure to get chocolate with the optimal properties without using tempering process. SANS was the unique tool which allows determining the nanostructure of phospholipids at low concentration in such a complex matrix (cacao butter and commercial chocolate) with a precise control in temperature[3-4]. Commercial deuterated phospholipids were used in both cocoa butter and commercial chocolate to get an insight on the nanostructures formed by phospholipids in these media in the molten state. Then, we followed what happened during cooling to room temperature.

2/Materials and methods

We used one model deuterated phospholipid, dimyristoylphosphatidylcholine (d-DMPC) with SLD of 8.11×10^{10} cm^{-2}. In a **first step**, the d-DMPC concentration was varied above CAC (from 0.1 to 0.5 wt.%) at a fixed temperature (80 °C) in cacao butter (SLD around 0.2×10^{10} cm^{-2}). This allowed us optimizing the concentration to be studied in the next steps and validating that the structures formed do not depend on concentration in this range. In a **second step**, one sample at the chosen d-DMPC concentration (0.5 wt.%) in cocoa butter and chocolate was measured at various temperatures. We decreased the temperature step

by step to determine the structural evolution of the nanostructures when the crystallization of triglycerides started to occur.

Samples were prepared by using commercial cocoa butter (scattering length density (SLD) of 0.211×10^{10} cm^{-2}), chocolate bought in the local supermarket (SLD of 0.211×10^{10} cm^{-2}), and deuterated d54-DMPC (Larodan, Sweden) with an SLD of $8.11x10^{10}$ $cm^{-2.}$ Samples were held in 1 mm optical path-length flat quartz cuvettes. SANS data on cocoa butter and chocolate were acquired on D22 at ILL. The wavelength was fixed at 6.0 Å (relative FWHM 10 %), the rear detector was at 17.6 m from the sample, and the front detector at 1.4 m with a 20° angle, thus covering a continuous Q-range of 2.3x10-3 - 0.64 Å-1. Data were reduced with the program Grasp v10.17, normalizing with monitor, subtracting the contribution from the empty cell, taking into account noise from the measurement with a sintered 10B4C piece at the sample position, and using for transmission the intensity from the attenuated direct beam. Parallax from the detector and from the sample attenuation were corrected for and a flat field was used. Absolute scale was obtained from the measurement of flux with a chopper having a well-defined attenuation coefficient. The temperature was controlled using an external circulating thermal bath at 80°C. The scattering of pure cocoa butter was measured separately.

3/Results

Please note that this report does not present exhaustively all results gathered during the experimental campaign Only the more representative results are presented and discussed here. Please see the 5 articles to see all the results:
https://doi.org/10.1016/j.jcis.2024.07.218
https://doi.org/10.1063/5.0196389
https://doi.org/10.1016/j.jcis.2024.05.213
https://doi.org/10.1021/acs.cgd.3c01130
https://doi.org/10.1021/acs.cgd.5c00575

We carried out SANS measurements on d_{54}-DMPC in cocoa butter at 0.5 wt.% (above CAC) and at 80 °C (above Krafft Temperature) and cooled to 25 °C after heating. Results are shown in Figure 1-A. At low Q, the Q^{-4} slope shows that residual aggregates of DMPC not well dispersed and still in crystalline form in small amounts were present. We focused on the medium and high Q region (from 10^{-2} to 0.6 $Å^{-1}$). To obtain quantitative values on the size and shape of the structures, we fitted the data to the form factor of a sphere (red line). Our results suggest a reverse micelle with a radius of 2.1 nm. This value is in accordance with the literature showing similar radius for phospholipids reverse micelles in vegetable oil. Upon cooling to 25 °C the CB crystalizes and no self-assembled structure can be seen at the low Q ranges (Figure 1-B). Higher Q values show the 001, 002 and 003 peaks of TAGs associated with Form V polymorphism CB. The experiment was conducted at other concentrations with similar results as seen in Figures 2-A and 2-B. The data suggests the presence of a self-assembled structure of DMPC in cocoa butter and more precisely of a DMPC reverse micelle when in a liquid phase and a disorganization or loss of self-assembled structures in the solid phase. It is interesting to notice that at lower DMPC concentration (0.1 wt.%), no self-assembled structures were detected, showing that DMPC concentration needs to be above 0.1 wt.% in CB to form such structures (Figure 2-A and 2-B). Then, in order to investigate the self-assembly of DMPC in a real system, we carried out the identical SANS measurements on d_{54}-DMPC in a commercial 90 % chocolate. We used d_{54}-DMPC at 0.5 wt.% (above CAC) and at 80 °C (above Krafft Temperature) and cooled to 25 °C after heating. In both of these cases, no self-assembly of deuterated DMPC could be identified from the data at high and low temperatures (Figures 1-C and 1-D). The cooled, and recrystallized sample displays the form V polymorphism CB signal from the 001, 002, and 003 reflections at higher Q values. The experiment was conducted at lower concentrations with similar results as seen in Figures 2-C and 2-D.

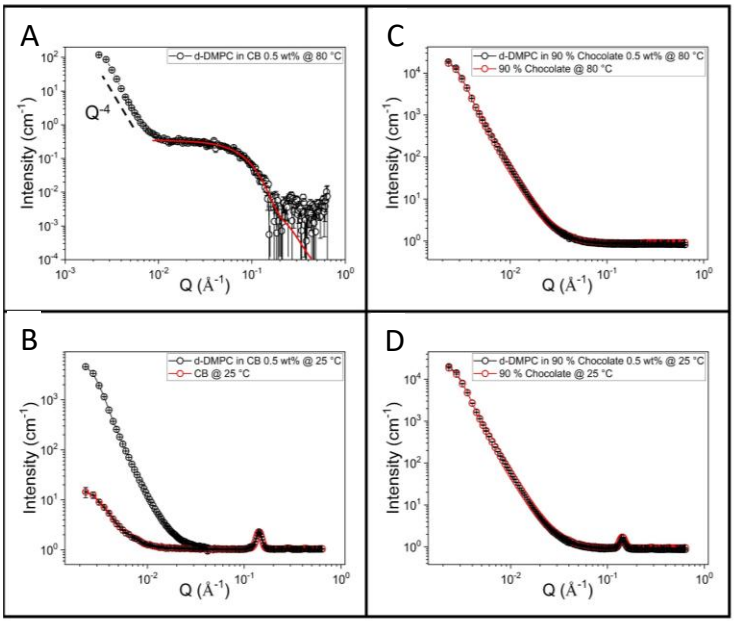

Fig 1. Black corresponds to azimuthally averaged SANS data for 0.5 wt.% of d54-DMPC in cocoa butter at 25 °C, and in 90 % chocolate at 80 °C and 25 °C. Red corresponds to azimuthally averaged SANS data for cocoa butter at 25 °C, 90 % chocolate at 80 °C and 25 °C.

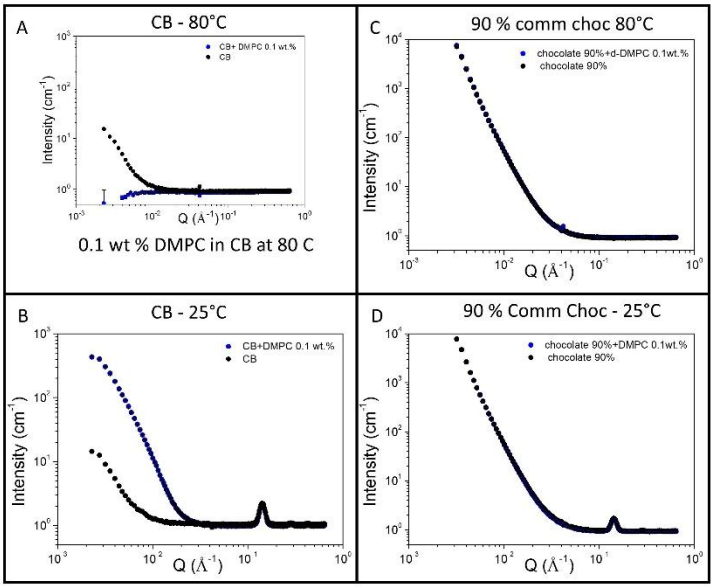

Fig 2. SANS spectra of pure cacao butter (black) and d-DMPC (0.1 wt.%) in cacao butter (blue) at: (A) 80 °C and (B) at 25 °C . SANS spectra of pure chocolate 90 % (balck) and d-DMPC (0.1 wt.%) in cholcolate 0 % (blue) at: (C) 80 °C and (D) 25°C

4/Conclusion

SANS studies suggested that DMPC forms a variety of micelles in cocoa butter. A strong interaction between the DMPC micelles and the triglyceride Palmitoyl-Oleoyl-Stearoyl glycerol (POS), the most abundant triglyceride in cocoa butter which also directs the triclinic crystallization of the cocoa butter, was also observed by SAXS, interfacial tension measurements and ATR-FTIR. This suggested that DMPC micelles serve as a seeding surface, templating form V crystal growth via its effects on POS. We propose a mechanism which involves a solid-state polymorphic transition form IV to V POS in the seeding crystals. Crystal strain and defects were observed in the templated nano and microstructure observed by synchrotron micro-computed tomography and SAXS. These could affect the product's properties, suggesting that a simple tempering step may still be required.

References:

1.Chaiseri, S. & Dimick, P. S. *J. Am. Oil Chem. Soc.* **72**, 1491–1496 (1995). https://doi.org/10.1007/BF02577842
2.Chen, J., Ghazani, S. M., Stobbs, J. A. & Marangoni, A. G. *Nat. Commun.* **12**, 1–9(2021). https://doi.org/10.1038/s41467-021-25206-1
3. Stobbs, J. A., Pensini, E., Ghazani, S. M., Leontowich, A. F., Quirk, A., Tu, K., ... & Marangoni, A. G. (2024). *Crystal Growth & Design*, *24*(7), 2685-2699. https://doi.org/10.1021/acs.cgd.3c01130
4. Stobbs, J. A., Ghazani, S. M., Tu, K., Pensini, E., Fameau, A. L., & Marangoni, A. G. (2025). *Crystal Growth & Design*. https://doi.org/10.1021/acs.cgd.5c00575