Experimental Report

Proposal: **9-13-484** **Council:** 10/2012

Title: Structure of Interfacial Layers Formed from Rupture of Oleosomes

This proposal is resubmission of: 9-13-446

Researh Area: Other...

Main proposer: **WASCHATKO Gustav**

Experimental Team: WASCHATKO Gustav
JACKSON Andrew
SCHIEDT BIRGITTA
MAURER Sania

Local Contact: CAMPBELL Richard

Samples: Oleosomes extracted from soybeans (protein and lipids)

Instrument	Req. Days	All. Days	From	To
FIGARO Langmuir trough	3	4	29/03/2013	02/04/2013

Abstract:

Soy foods have rising popularity in European countries, due to ecological or health benefits, lactose intolerance or allergies against milk proteins. Traditional soy food such as soymilk, tofu or yuba contains small oil droplets, which have been known for many years in botany as oleosomes or oil bodies. These oleosomes are a natural oil-in-water emulsion formed from an "oil phase" of triacylglycerides with an "emulsifier" consisting of phospholipids and unique umbrella shaped proteins called oleosins which stick into the oil phase. The exterior hydrophilic part of the oleosomes (N- and C- terminal domains) shields the phospholipids and is, because of its pH-dependent charge, responsible for the extraordinary stability of oleosomes against coalescence and creaming. At a clean air-water interface oleosomes show an interesting behavior, including their time and concentration dependent rupture followed by the formation of a stable 2D-film of its constituents. This film consisting of TAGs, phospholipids and oleosins is different in its composition according to the subphase conditions, time and surface area (compression).

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Structure of Interfacial Layers Formed from Rupture of Oleosomes

Instrument: FIGARO

Date: 29/03/13 to 02/04/13

Introduction

In oleogenius plants, like soybean and sunflower, neutral lipids are stored in abundant, sub-cellular organelles, called oil bodies or oleosomes. By gentle aqueous extraction, intact oleosomes can be obtained in form of a natural oil-in-water emulsion. The oil droplets, mainly consisting of triacylglycerides (TAG), are surrounded by a monolayer of phospholipids (PL) in which umbrella-like shaped proteins, called oleosins, are embedded. Oleosins are unique to plant seeds and mainly responsible for the remarkable stability of the oleosomes against aggregation and coalescence. They are anchored in the oil matrix by a long hydrophobic domain. The oleosome surface is completely covered by their amphiphilic N-and C-termini, keeping the oil droplets through steric hindrance and electrostatic repulsion apart from each other [1, 2]. At a clean air-water interface oleosomes show an interesting behaviour, including their time and concentration dependent rupture followed by the formation of a stable 2D-film of its constituents. This film consisting of TAG, PL and oleosins is different in its composition according to the subphase conditions, time and surface area (compression) [3].To date, the kinetic mechanism of the rupture, as well as the orientation, conformation and arrangement of the oleosins within the 2D-film is not fully understood.

Therefore, we aim at investigating the time- and pH-dependent changes in the monolayer composed of the oleosome constituents by reflectometry to correlate them with the adsorption kinetics and breakdown of the oleosomes at the air-water interface. The role of the oleosin is elucidated by comparing the native oleosomes with enzymatically digested oleosomes, where the hydrophilic domains of the oleosin have been cleaved. Film balance measurements performed simultaneously to the reflectivity experiments allow us to monitor the surface pressure.

Experimental

First, 3.3 mg/L of native oleosome were inserted into the subphase of pure D_2O (160 mL) provided in a Langmuir trough. However, the inhomogeneous diffusion of oleosomes to the air-water interface resulted in significant off-specular scattering, why premixed solutions of oleosomes were prepared instead. A test range of different oleosome concentrations (8, 80, and 800 µl with 14.67 % of oleosomes respectively) at two different contrasts (pure D2O and ACWM, the latter adjusted to pH 8 and 10 mM) was performed to evaluate the most suitable one, which proofed to be 80 µl. Subsequently, premixes of 80 µl native oleosomes and oleosomes with enzymatically digested oleosins were measured at the two different contrasts over a time of 8 h. Simultaneously, changes in the surface pressure were monitored over time. Runs were probed at three different angles; $A_1 = 0.624°$, $A_2 = 1.98°$, and $A_3 = 3.78°$.

Results

The surface pressure measurements recorded in parallel to the neutron reflectivity are shown in Figure 1. Premixes of native oleosomes showed a lower surface pressure than premixes of digested oleosomes, respectively at both contrasts. As this correlates with our results from a previous work [3], it confirms that a premixed oleosome solution is suitable to study the adsorption and film formation kinetics of oleosome ruptured at the air-water interface with neutron reflectivity.

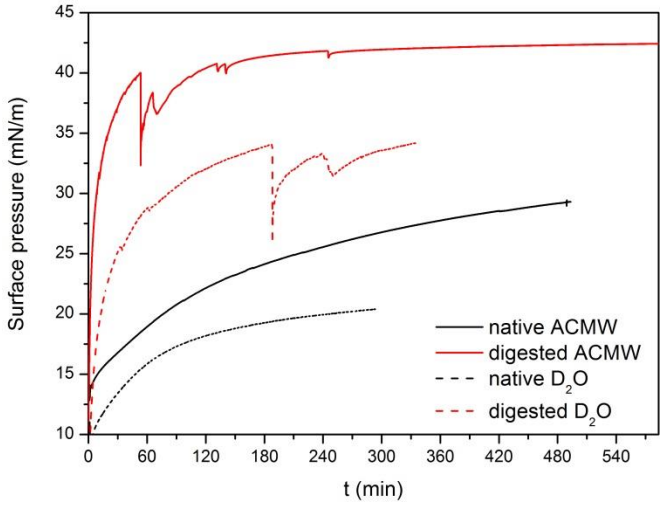

Figure 1: Surface pressure versus time for native and digested oleosomes in ACMW and pure D_2O

The surface pressure was always lower for the oleosomes in D_2O than in ACMW. The reason for this is not yet clear.

The corresponding neutron reflectometry data are shown in Figure 2a and 2b for native and digested oleosomes, respectively. It is clearly visible, that for native oleosomes the interfacial film is undergoing stronger changes with time than for the digested oleosomes. We attribute this to the presence of oleosins in the native oleosomes.

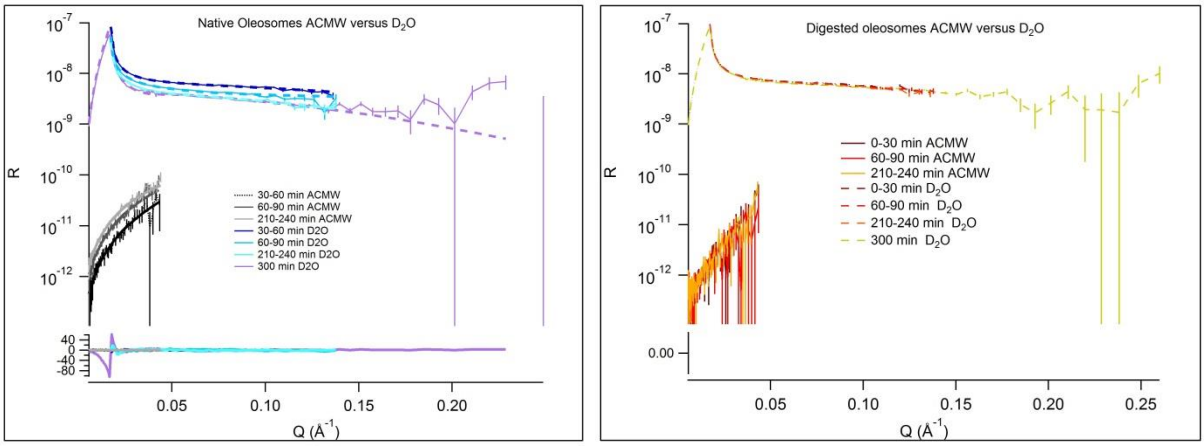

Figure 2: Reflectivity recorded at different times for a) native and b) digested oleosomes in ACMW and D_2O

Preliminary fits of the data for native oleosomes are shown in Figure 2a and their corresponding SLD profiles and fitting coefficients in Figure 3 and Table 1, respectively. A one-layer model has been used.

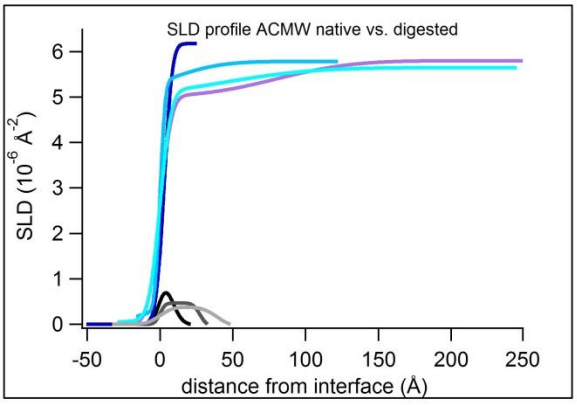

Figure 3: SLD profiles for native oleosomes in ACMW (grey curves) and D_2O (blue curves)

Point	coef_run08A_01_f	coef_run08A_03_f	coef_run08A_08_f	coef_run10C_01_f	coef_run10C_03_f	coef_run10C_08_f	coef_run10_R	Coefficients
2	0	0	0	0	0	0	0	fronting-SLD
3	-0.0153899	-0.0400477	-0.0574949	6.17813	5.78307	5.64597	5.79923	backing-SLD
4	1.53785e-06	1.34531e-06	1.99079e-06	6.8045e-09	1.04145e-08	1.40063e-08	8.08249e-09	bkg
5	6.91661	3.80081	8.12146	4.37452	27.9603	49.1809	41.0187	backing-roughnes
6	4.06202	27.4837	39.2824	2.87478	5.53274	43.4588	80.0758	1-thickness
7	2.26066	0.466166	0.377922	0.0561022	4.99669	4.99274	4.99815	1-SLD
8	0	0	0	0	0	0	0	1-solvent
9	4.65281	2.954	6.97542	11.3705	2.81993	6.08087	6.35168	1-roughness

Table 1: Coefficients resulting from the preliminary fits of the data for native oleosomes in ACMW (Run08) and D_2O (Run10)

As a general trend it can be said that as expected, the excess surface and probably thickness increase with time for both contrasts. However, due to the complex multi-component nature of the ruptured oleosomes (proteins, PL, TAG) a more precise determination of the fitting parameters and underlying model is difficult to achieve. Therefore, complementing measurements with simplified systems (TAG, PL, PL stabilised emulsion, reconstituted oleosomes) are planned.

References

[1] Hsieh, K.; Huang, A. H. C., Endoplasmic Reticulum, Oleosins, and Oils in Seeds and Tapetum Cells. *Plant Physiology* **2004,** 136, (3), 3427-3434.

[2] Iwanaga, D.; Gray, D. A.; Fisk, I. D.; Decker, E. A.; Weiss, J.; McClements, D. J., Extraction and Characterization of Oil Bodies from Soy Beans: A Natural Source of Pre-Emulsified Soybean Oil. *Journal of Agricultural and Food Chemistry* **2007,** 55, (21), 8711-8716.

[3] Waschatko, G.; Junghans, A.; Vilgis, T. A., Soy milk oleosome behaviour at the air-water interface. *Faraday Discussions* **2012**.