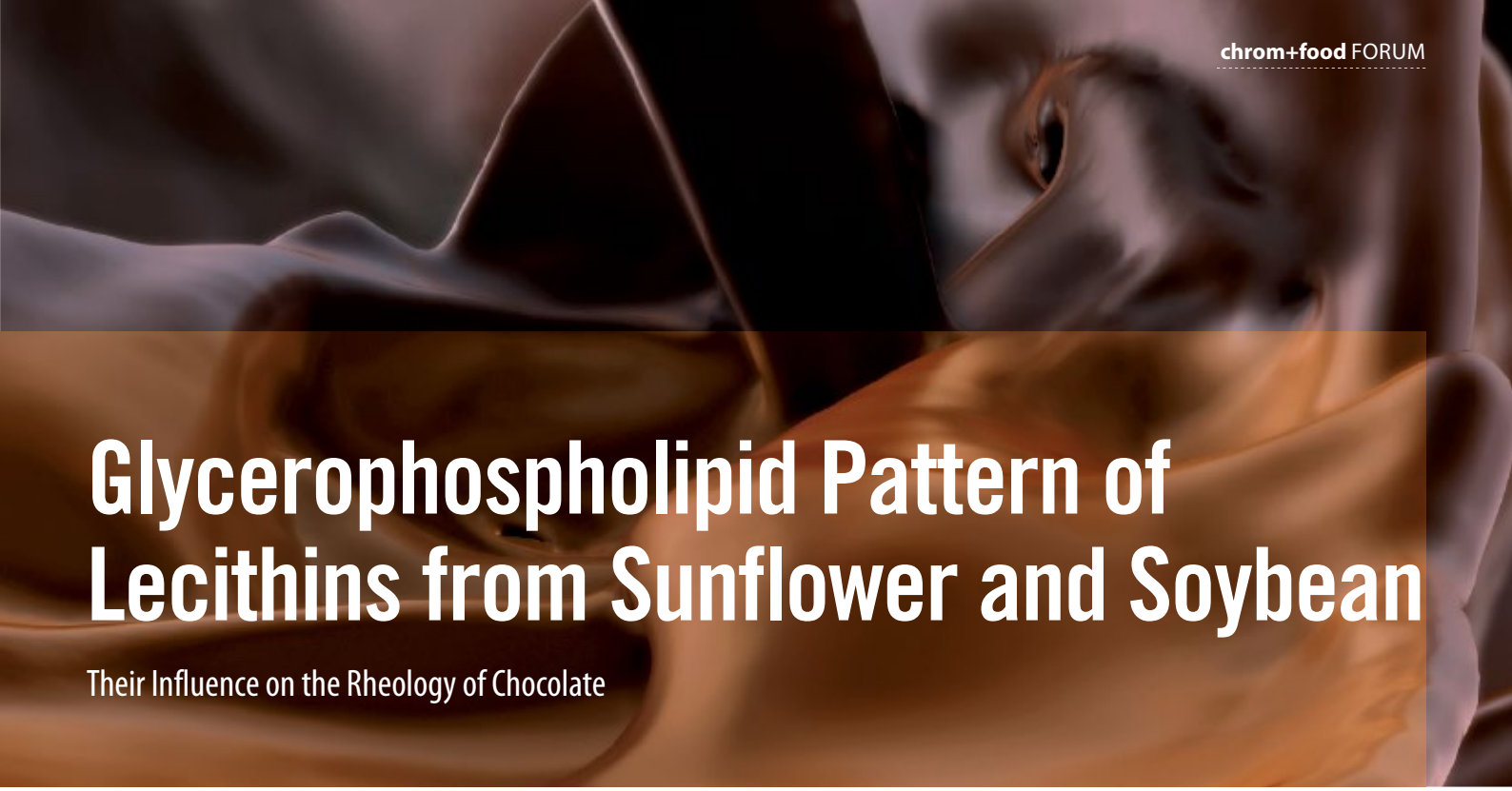

Glycerophospholipid Pattern of Lecithins from Sunflower and Soybean

Their Influence on the Rheology of Chocolate

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As natural emulsifier and food additive (E 322) lecithin plays a significant role in the production of various foodstuffs such as chocolate.

Comprising of polar and nonpolar lipids (~50% glycerophospholipids and ~34 % triacylglycerides), especially the glycerophospholipids therein play an important role in the production of chocolate. Their influence on the rheological parameters of molten chocolate (miscibility of continuous and dispersive phase) is crucial for receiving the desired product. [1-3] Different glycerophospholipids can affect the rheology in various ways. Phosphatidylcholine (PC) for example affects the reduction of the viscosity, while phosphatidylethanolamine (PE) reduces the chocolate yield value. [3, 4] The commonly used soybean lecithin has several shortcomings such as the necessity of declaration on the packaging due to intrinsic allergens or the difficulty in acquiring the lecithin from non-genetically modified sources. Therefore, the substitution with another source that has a relatively comparable glycerophospholipid pattern, like e.g. sunflower, seems to be the logical next step. [5]

As a fast and easy method, high-performance thin-layer chromatography (HPTLC) is suitable for the qualitative and quantitative analysis of crude lecithin samples. The matrix robustness of HPTLC allows simple sample preparation like dissolvation in an appropriate solventand centrifugation. On a 20 x 10 cm plate up to 20 tracks can be applied, meaning it is possible analyze up to eight samples in duplicate and in parallel, with 4 tracks left for the standard calibration. After the automated chromatographic separation the visualization can be easily done with primuline, a relatively non-toxic reagent. Under UV-light at 366 nm sharp, well-separated zones become visible and allow easy and fast visual qualitative as well as densitometric quantitative evaluation (Fig. 1). By comparing the hRF values of a standard substance with zones found in the sample, it is easy to assign it to a corresponding standard compound.

Moreover, the visual comparison and evaluation allows getting quick first impressions to what extent the individual lecithin samples differ. In this example, three commercially available crude lecithin extracts, two from sunflower and one from soybean, were analyzed. While major differences were not visible at first glance, both sunflower lecithin samples (SN1, SN2) showed a significant lower PE content compared to the soybean lecithin (SJ). [6]

For a further and more detailed characterization of the different lecithins, it is also possible to take a look at the glycerophospholipids 'fatty acyl pattern via electrospray mass spectrometry (ESI-MS). Owed to the TLC-MS Interface the process is fast and uncomplicated. Zones of interest are marked with a soft pencil and then can be directly eluted into the MS, with the time required being less than five minutes. For the three lecithin samples, it was clearly shown that all three extracts had a comparable fatty acyl pattern regardless of whether they originated from soybean or sunflower (Fig. 2). [6]

Another option for their characterization is the investigation via matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOFMS). An appropriate adapter target for HPTLC plates (5 x 7.5 cm) allows the hyphenation HPTLC-MALDI-TOFMS (Fig. 3A). Aluminum plates, pre-cut to this small format, are commercially available. The deposition of the MALDI matrix is carried out by repeated dipping and drying of the plate into the matrix solution (e.g., 2,5-dihydroxybenzoic acid). For the glycerophospholipids in this example, the resulting mass spectra (Fig. 3B) were comparable with the mass spectra obtained via HPTLC-ESI-MS. The recording of mass spectra via high-resolution TOFMS allowed verification and validation of the results obtained by ESI-MS. [6]

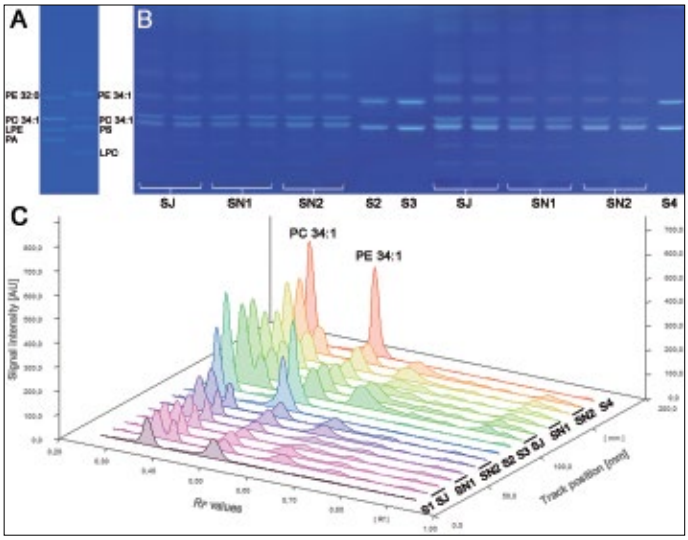

Fig. 1: Shown are (A) different glycerophospholipid standards and (B) PC and PE standards applied in different volumes (S2-S4) beside lecithin samples of soybean (SJ) and sunflower (SN1, SN2) as well as (C) a HPTLC 3D densitogram. Reprinted with permission from [6]. © 2015 American Chemical Society

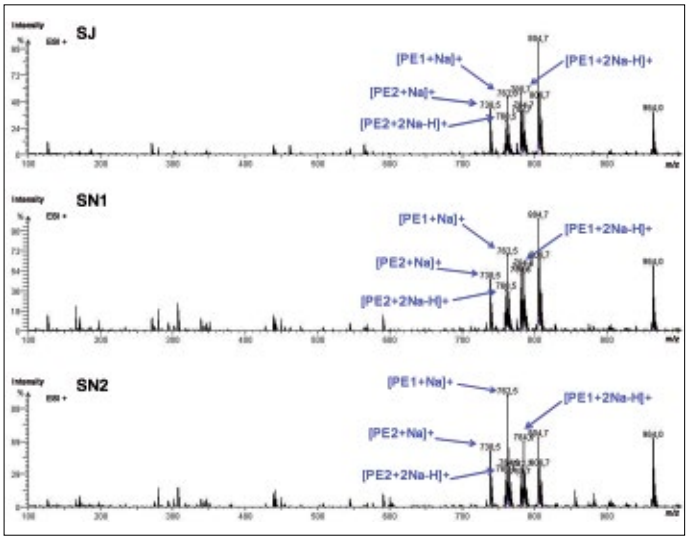

Fig. 2: Identification of the samples' PE zones (hR_f42) via HPTLC-ESI^+-MS after zone elution with the TLC-MS Interface. Reprinted with permission from [6]. © 2015 American Chemical Society

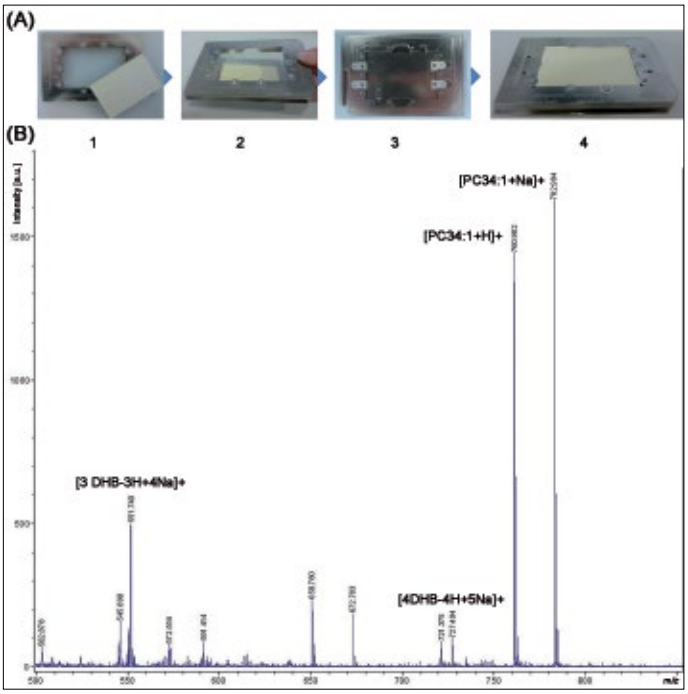

Fig. 3: Shown are (A) how to place the HPTLC foil in the TLC-MALDI adapter target and (B) a HPTLC-MALDI-TOFMS spectrum of PC. Reprinted with permission from [6]. © 2015 American Chemical Society

Further studies into the rheology (yield value and viscosity) showed that when adding the three lecithin samples to chocolate masses (white, milk, and dark) according to the original receipt, the deviations in the rheology were negligible. [6]

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