HPTLC-FLD-ESI-MS and HPTLC-MALDI-TOF/TOF MS analysis of lecithins used in the production of chocolate

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Introduction

Lecithin or rather phospholipids are added as emulsifier E322 to various products in food industry [1]. Most of the lecithin used in chocolate production (Fig. 1) is obtained from soybeans requiring declaration with regard to intrinsic allergens and GMOs. Efforts have been made to use non-genetically modified sun-flower lecithin instead. Phospholipids are crucial for the miscibility of the different components and stability of the chocolate mass. Of special interest were phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Both phospholipids influence the rheology of molten chocolate differently. While PC reduces viscosity, PE is more effective reducing the rheological *Casson* yield value than the viscosity. The lecithin samples were quantitatively analyzed for their phospholipid content and fatty acid pattern by HPTLC-FLD/MS as well as for their rheological behavior [3].

Fig. 1 Liquid lecithin, liquid chocolate mass and different types of chocolate bars [2]

Results and discussion

After minimal sample preparation, *i.e.* extraction with methanol and *i*-propyl acetate 2:3 (v/v) and centrifuging, the phospholipids of three lecithin samples (two sunflower lecithins, one soy lecithin) were separated with chloroform – methanol – water – ammonia (25 %) 30:17:2:1 (v/v/v/v) [4]. The plates were derivatized with a 0.05 % primuline reagent (w/v) and documented (Fig. 2). PC and PE in the lecithin samples were quantified after fluorescence measurement at 366/>400 nm.

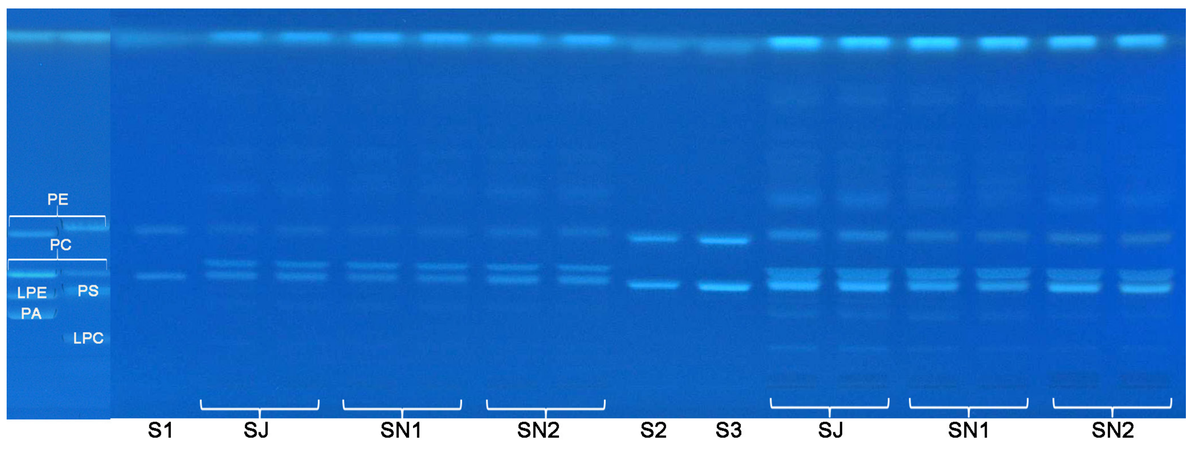

Fig. 2 HPTLC chromatograms of all phospholipid standards (first two tracks) and of PC (34:1) and PE (34:1) with different volumes applied (S1-S3) for quantitation beside samples of soy lecithin (SJ) and sunflower lecithins (SN1 and SN2)

PC and PE were the main components in lecithins from both sources. While the visual phospholipid fingerprint was similar for all three samples, quantitative analysis showed differences in soy and sunflower lecithin samples concerning their PC and PE content. Both sunflower lecithin samples showed significantly lower PE values than soy lecithin. The PC content of soybean and sunflower lecithins was similar (20 ± 2 mg/g), but the PE content ranged between 14 to 22 mg/g. This difference was reflected in the PE/PC ratio. For soybean lecithin the ratio was <1, while the ratio for the sunflower lecithin samples was >1.

For characterization of the molecular species, the sample zones of PE and PC were analyzed via HPTLC-ESI MS (expression CMS, Advion) and HPTLC-MALDI-TOF/TOF MS (Ultraflex 1, Bruker Daltonics). For all three lecithin samples, the PC and PE mass signal patterns were comparable. By HPTLC-ESI-MS two PC mass signals at *m/z* 782 $[PC1]^+$ and 785 $[PC2]^+$ and their respective sodium adducts at *m/z* 804 $[PC1+Na]^+$ and 780 $[PC2+Na]^+$ were identified, suggesting two molecular species: PC 36:4 (PC1) and PC 34:2 (PC2). A further mass signal at *m/z* 564 suggested fragmentation, *i.e.* elimination linoleic acid $[PC1-C18:2]^+$ and palmitic acid $[PC2-C16:0]^+$.

For PE, two molecular species could be identified: PE 36:4 (PE1) and PE 34:2 (PE2). Mass signals of their mono- and disodium adducts were assigned. In summary, dilinoleoyl and palmitoyl-linoleoyl derivates of PE and PC could be identified corresponding with literature. These results were confirmed by HPTLC-MALDI-TOF/TOF MS (Fig. 3).

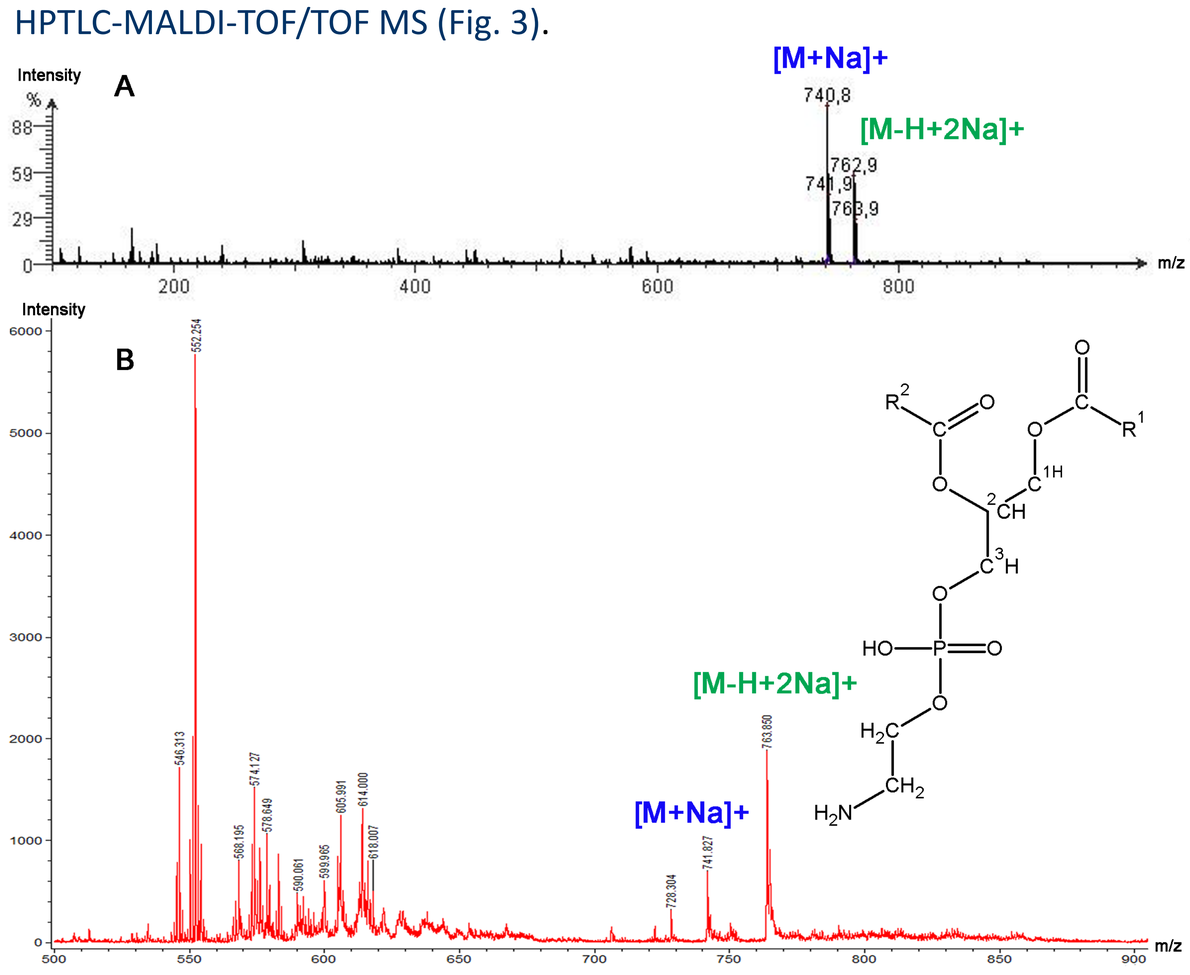

Fig. 3 Mass spectra of PE (34:1) obtained by HPTLC-ESI$^+$ MS (A) and HPTLC-MALDI-TOF/TOF MS (B)

Rheology of the respective chocolates using sunflower or soybean lecithins showed overall comparable results. Slightly higher viscosity was measured for dark chocolate when using SN1 instead of SJ. When using SN2 instead of SJ the rheological yield value of milk chocolate was slightly increased. No significant differences in flow parameters were detected for white chocolate. When comparing the PE and PC contents to the rheological results, no causal correlation between the PE content of the lecithin and the yield value in any of the used chocolates could be found. In addition, neither the lower PC content of sunflower lecithin 1 nor the higher amount of PC in sunflower lecithin 2 compared to soybean lecithin had a divergent effect on the viscosity of dark or milk chocolate. Only for white chocolate, a negative correlation (r = -0.99) was indicated. Finally, the differences in PC and PE content in the three lecithin samples were not significantly the reason for the slight differences in the rheology of milk or dark chocolate.

Conclusions

HPTLC proved to be well suited for the investigation of phospholipids in crude lecithin extracts. It allowed a time- and cost-effective quantitative analysis of phospholipids as well as their characterization by hyphenation with two mass spectrometric methods.

References [1] Verordnung über die Zulassung von Zusatzstoffen zu Lebensmitteln zu technologischen Zwecken (Zusatzstoff-Zulassungsverordnung, ZZulV), 2012. [2] Sources: www.gayatriglobal.net, www.besserhaushalten.de, www.planet-wissen.de [3] S. Krueger, L. Buermann, G. Morlock, Comparison and characterization of soybean and sunflower lecithins used for chocolate production by HPTLC-FLD-ESI-MS, in submission. [4] E. Reich D. Handloser, V. Eidmer, J Liq Chromatogr Relat Technol 31 (2008) 1857-1870.

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