^{31}P NMR spectroscopy – strong interaction between wild **bilberry** components and **cell membranes**

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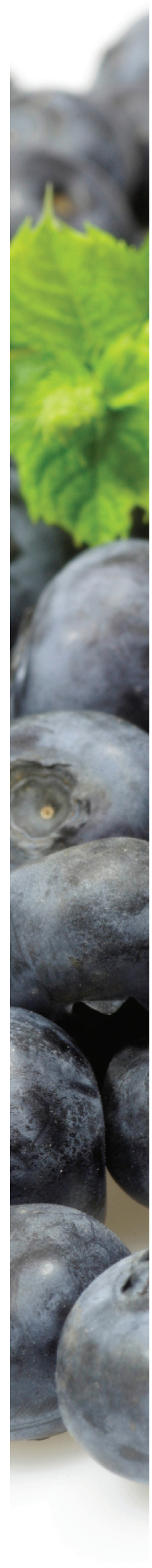

Introduction

A high intake of berries is considered healthy. These beneficial effects are often ascribed to their antioxidant properties. However, there is no evidence that a high intake of pure antioxidants promotes health, and the mechanism behind their bioactivity remains unclear. Thus, the aim of this study was to examine how interactions between berry components and cell membranes could potentially contribute to explain the **health benefits of berries**.

Methods

Berry samples from a large variety of berries were collected. The methanol-water phase from a dual-phase extraction procedure of each berry sample was added to different model-membrane systems. All NMR experiments were performed at 310K.

NMR methods:

- Solution-state ^{31}P NMR - DPC (dodecylphosphocholine) micelle model system. Bruker Avance III 600 MHz spectrometer with a 5mm TXI probe. Lyophilized berry extract was solubilized in MeOH and mixed with 150 mM DPC in a 0.75 M Pi-buffer (pH=7.4).
- Oriented ^{31}P Solid-State NMR - Mechanically aligned samples of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine). Mass ratio 20:1 (POPC : berry extract). Bruker- 400 Avance wide-bore spectrometer with a flat-coil probe.
- Static and MAS ^{31}P NMR – POPC multilamellar vesicles (MLVs). Bruker- 400 Avance wide-bore spectrometer with a 4 mm CP-MAS probe. Mass ratio 5:1 (POPC : berry extract). DMFIT software was used for simulation.

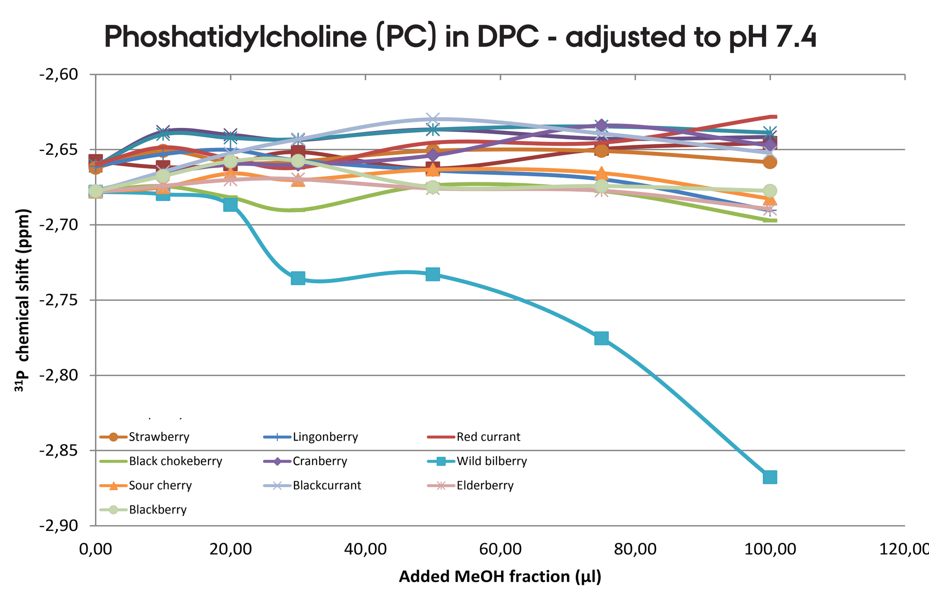

Fig. 1 Solution-state ^{31}P NMR

Table 1. ^{31}P chemical shift of the POPC peak, CSA width and the principal components of the ^{31}P chemical shift tensor, isotropic POPC peak.

Experiment	Flatcoil	Static				MAS
Sample	Oriented POPC peak (ppm)	CSA width (ppm)	δ_{11} (ppm)	δ_{22} (ppm)	δ_{33} (ppm)	δ_{iso} (ppm)
Pure POPC	31.09	44.20	28.03	-15.87	-16.16	-0.67
Aronia	31.42	45.52	28.48	-14.10	-17.03	-0.66
Blackberry	31.36	45.61	29.59	-14.53	-16.03	-0.77
Blackcurrant	30.45	43.98	27.47	-13.68	-16.52	-0.69
Common sea-blackthorn	31.77	44.64	27.80	-14.53	-16.85	-0.69
Cranberry	31.00	42.99	27.24	-14.90	-15.75	-0.72
Elderberry	31.44	43.63	27.37	-15.11	-16.26	-0.70
Lingonberry	30.92	43.21	25.14	-12.67	-18.07	-0.76
Raspberry	31.32	43.31	27.65	-14.24	-15.66	-0.72
Redcurrant	29.55	43.16	27.29	-14.50	-15.87	-0.72
Sour cherry	32.24	41.88	26.02	-15.06	-15.85	-0.65
Strawberry	31.35	44.70	28.46	-14.27	-16.25	-0.67
Wild bilberry	30.18	49.14	30.22	-12.28	-18.92	-0.90
Wrinkled rose	31.72	43.13	27.31	-15.24	-15.81	-0.67

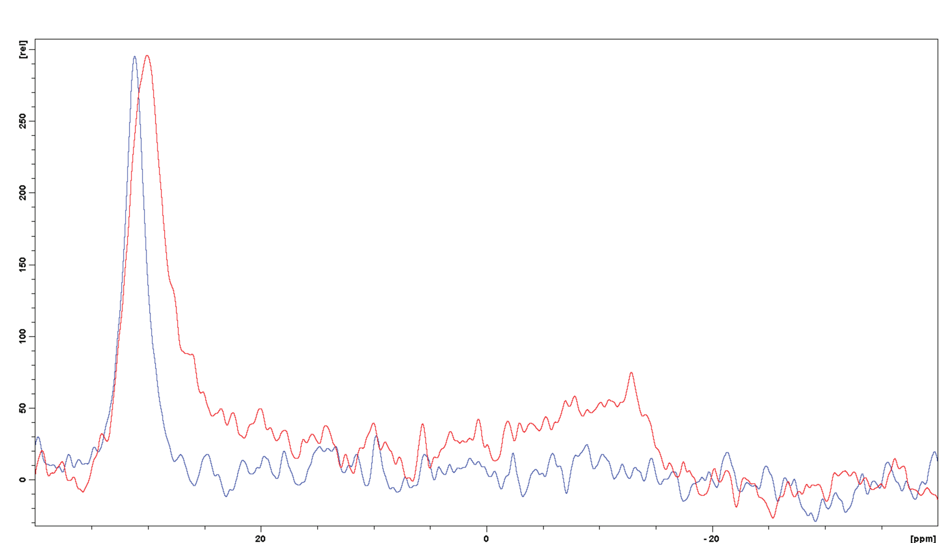

Fig. 2 MAS ^{31}P NMR. Blue: pure POPC. Red: wild bilberry.

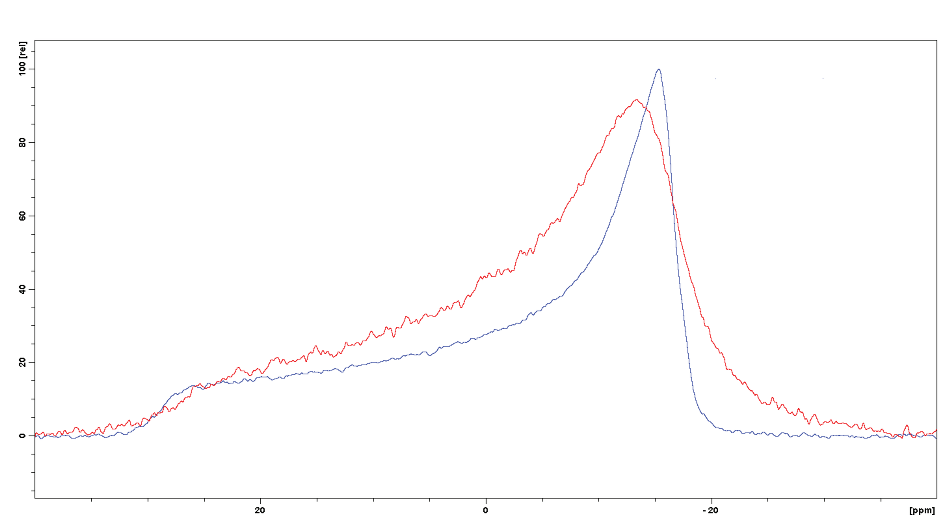

Fig. 3 Static ^{31}P NMR. Blue: pure POPC. Red: wild bilberry.

Results

NMR methods were applied to characterize the perturbations of the phospholipid headgroup of cell membranes by measuring the ^{31}P chemical shift

- Addition of wild bilberry extract resulted in changes in the ^{31}P chemical shift of the DPC micelles, which was not found for the other berry types (Fig. 1).
- Wild bilberry extract was the most significant modulator POPC bilayer (Fig. 2 and Table 1).
- Static and MAS ^{31}P NMR experiments with POPC MLVs revealed major changes with wild bilberry extract (Fig. 3).

Discussion/Conclusion

It is difficult to explain these major changes by addition of wild bilberry extract to changes of the axial rotational motional averaging of the lipids, but more likely it is due to alterations of the vesicle composition in the samples. Supporting the NMR findings, dynamic biophysical measurements of the membrane fluidity in liposomes and jurkat cells showed that wild bilberry was one of the most potent berry extracts in decreasing membrane fluidity at both the surface level and in the hydrophobic core of the membrane.

Overall, wild bilberry extract has a strong perturbation of cell membranes, which suggests that intake of **wild bilberry could potentially have the most health beneficial effect** compared to other berry types. Thus, more studies are needed to investigate the mechanism behind the bioactivity of wild bilberry and its health effects.

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