Future-proofing berryfruit
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Effects of polyphenol-rich berry extracts on human erythrocytes

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Background

Berries contain several bioactive compounds that can protect against oxidative stress.

A cellular model may complement chemical analysis and reveal different aspects of protection from complex foods.

Erythrocytes play a critical role in the antioxidant protection of the blood.

Erythrocytes are uniform, anucleate, easily accessible and lack the capacity for protein synthesis – thus limiting their responses to certain defined enzymatic processes.

Erythrocytes are anaerobic cells that lack mitochondria which limit their internal production of free radicals.

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Variation in atioxidant capacity (FRAP mmol/100 g dw) between and within species

Växtslag	Species	Samples (*n*)	Min	Average	Max
Slånbär	sloe, blackthorn (*Prunus spinosa*)	4	119,2	**141,2**	186,5
Nypon	rose hips (*Rosa*sp)	66	44,7	**101,0**	159,5
Aronia	purple chokeberry (*Aronia x prunifolia*)	2	77,2	**82,3**	87,4
Rosenkvitten	Japanese quince (*Chaenomeles japonica*)	17	34,9	**47,5**	60,4
Fläder	elderberry (*Sambucus nigra*)	3	38,0	**47,1**	53,7
Björnbär	blackberry (*Rubus* sp)	9	30,7	**45,4**	67,6
Blåbär	bilberry / blueberry (*Vaccinium* sp)	8	20,2	**41,8**	61,9
Lingon	cowberry (*Vaccinium vitis-idaea*)	4	26,9	**32,3**	35,9
Röda vinbär	red currant (*Ribes rubrum*)	6	25,9	**28,5**	31,0
Hallon	raspberry (*Rubus idaeus*)	17	14,2	**22,6**	31,7
Jordgubbe	strawberry (*Fragaria x ananassa*)	12	18,4	**22,6**	34,5
Svarta vinbär	black currant (*Ribes nigrum*)	6	17,7	**22,1**	32,3
Äpple (skal)	apple (skin!) (*Malus x domestica*)	6	14,0	**18,6**	23,9
Havtorn	sea buckthorn (*Hippophae rhamnoides*)	8	13,3	**18,3**	23,3
Rönnbär	rowanberry (*Sorbus* sp)	6	11,0	**17,7**	27,6
Krusbär	gooseberry (*Ribes uva-crispa*)	4	7,4	**10,8**	13,9
Plommon	plums (*Prunus domestica*)	6	8,3	**9,9**	12,2
Apelsin	orange (*Citrus* sp)	1	7,3	**7,3**	7,3
Vindruva	grape (*Vitis* sp)	3	1,5	**3,5**	6,4

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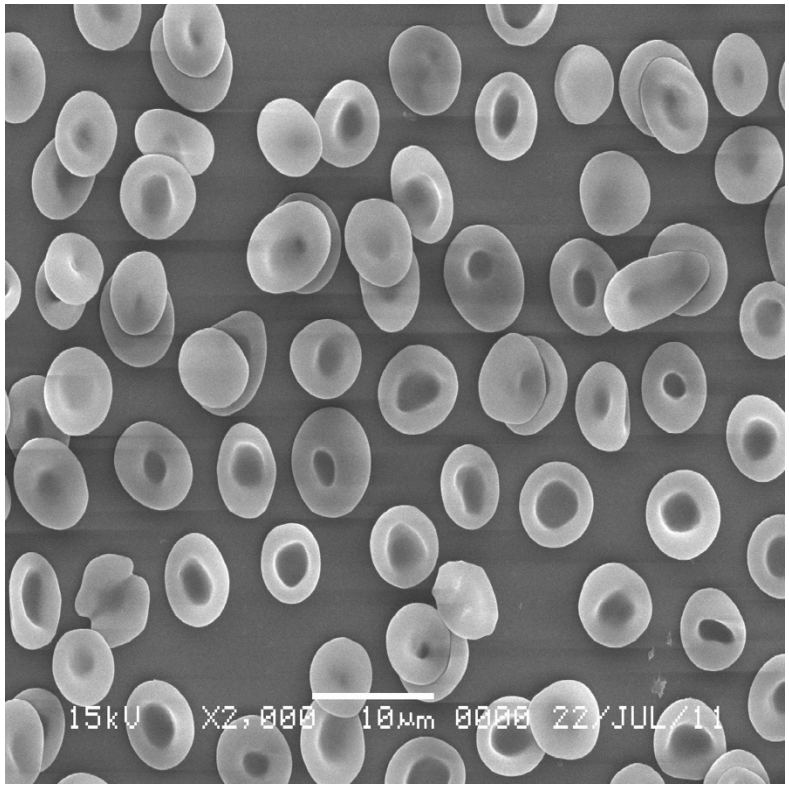
Objective

To study the protective effects of sequencial extracts of different berry species against oxidative stress in an erythrocytic assay as well as in an iron reduction assay (FRAP).



Picture: Erythrocytes prepared with SagM and fixed with glutaraldehyde diluted with SagM solution.

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Materials & Methods

Berries were sampled at full maturity:

- Black currants (*Ribes nigrum* 'Jadrenaja')
- Rose hips (*Rosa* 'BRo 484')
- Sea buckthorn berries (*Hippophae rhamnoides* 'Gibrid Pertjik')
- Strawberries (*Fragaria* x *ananassa* 'BFr 0121')
- Bilberries (*Vaccinium myrtillus*)
- Elderberries (*Sambucus nigra*)
- Lingonberries (*Vaccinium vitis-idaea*)

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Material & Methods

Berries were lyophilized, milled and extracted with:

1. heptane
2. ethyl acetate
3. ethanol
4. distilled water

Solvents were removed in a rotary evaporator (temperature below 30°C)

Dry extracts were dissolved in DMSO, diluted in SagM and stored frozen until analysis.

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Material & Methods

Berry extracts were mixed with purified human erythrocytes and incubated at 37°C for 60 min.

Erythrocytes were washed twice in SagM to remove any extracellular potential micronutrients.

Erythrocytes were lysed (by dH2O) and treated with a fluorescent dye which become fluorescent as a result of oxidative damage.

Free radicals were added as hydrogen peroxide and the fluorescence intensity was recorded after 10 min.

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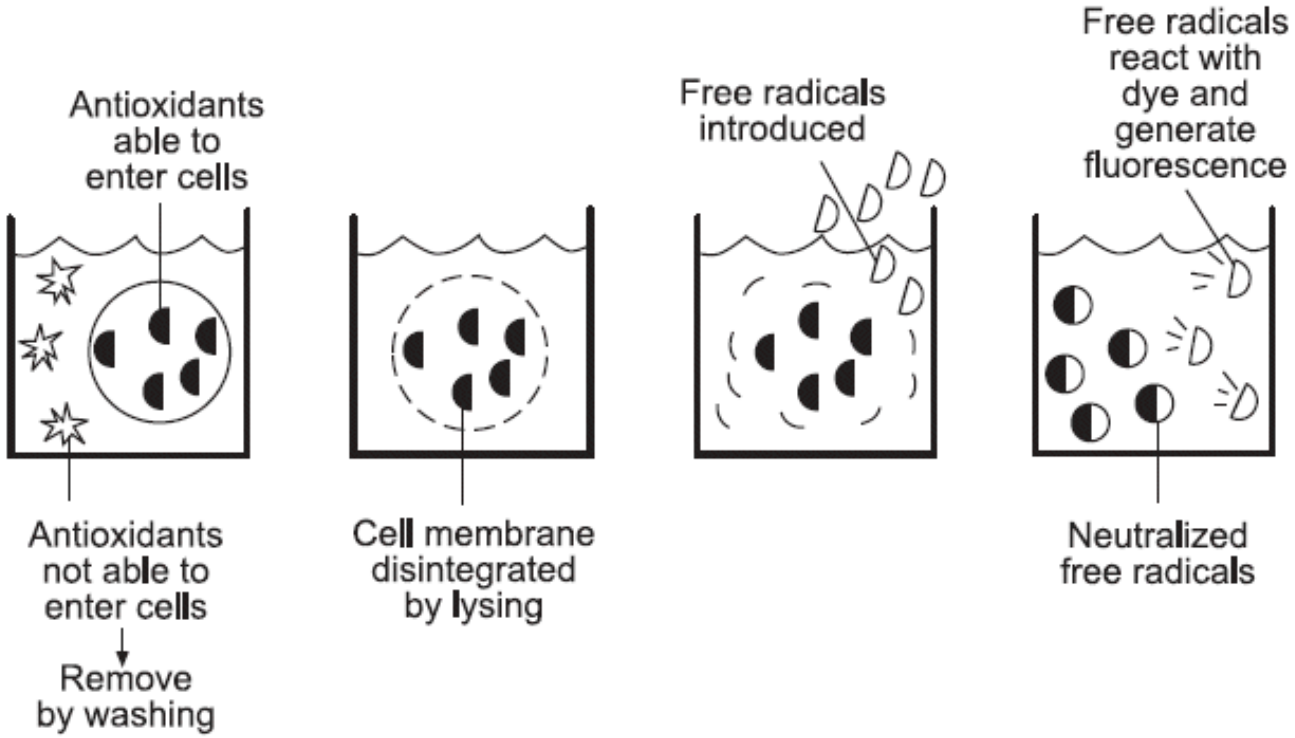
The erythrocyte assay



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Material & Methods

Additional analyses:

FRAP antioxidant capacity

Total phenols

Ascorbic acid

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Results: Ethylacetate extract

Plant material	Erythrocyte protection (%)	Total FRAP (µmol Fe2+/L)	Ascorbate FRAP (µmol Fe2+/L)
Bilberry	**42.4**	**463.1**	**1.6**
Black currant	3.3	3.2	0.2
Elderberry	-2.3	8.7	0.0
Lingonberry	-0.5	3.9	0.0
Rose hip	-6.1	10.2	0.1
Sea buckthorn	**51.9**	**5.5**	**3.6**
Strawberry	11.7	6.3	0.0

Widén et al. 2012a

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Results: Ethanol extract

Plant material	Erythrocyte protection (%)	Total FRAP (µmol Fe2+/L)	Ascorbate FRAP (µmol Fe2+/L)
Bilberry	3.4	16	0.0
Black currant	**36.6**	**54.3**	**28.9**
Elderberry	21.0	69.3	0.7
Lingonberry	3.1	82.7	0.7
Rose hip	-14.8	392.2	230.7
Sea buckthorn	**67.3**	**42.5**	**30.2**
Strawberry	5.1	49.6	12.2

Widén et al. 2012a

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Results: Water extract

Plant material	Erythrocyte protection (%)	Total FRAP (µmol Fe2+/L)	Ascorbate FRAP (µmol Fe2+/L)
Bilberry	-11.3	184.3	0.0
Black currant	**50.3**	**274.9**	**59.9**
Elderberry	7.6	482.0	0.0
Lingonberry	12.4	152.8	0.0
Rose hip	**-11.7**	**1760.2**	**824.9**
Sea buckthorn	**66.4**	**87.4**	**57.4**
Strawberry	-20.7	118.9	0.0

Widén et al. 2012a

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Results summary

Heptane extracts – no effect

All sea buckthorn extracts showed a superior protection of erythrocytes

Bilberry ethyl-acetate extracts, and ethanol and water extracts of blackcurrants, also protected the erythrocytes from oxidation

In contrast, all rose hip extracts and the water extracts of bilberries and strawberries showed pro-oxidant activity.

No correlation between the protective effects of berry extracts and total antioxidant capacity (FRAP) with one exception: ethyl acetate extracts and ascorbate FRAP

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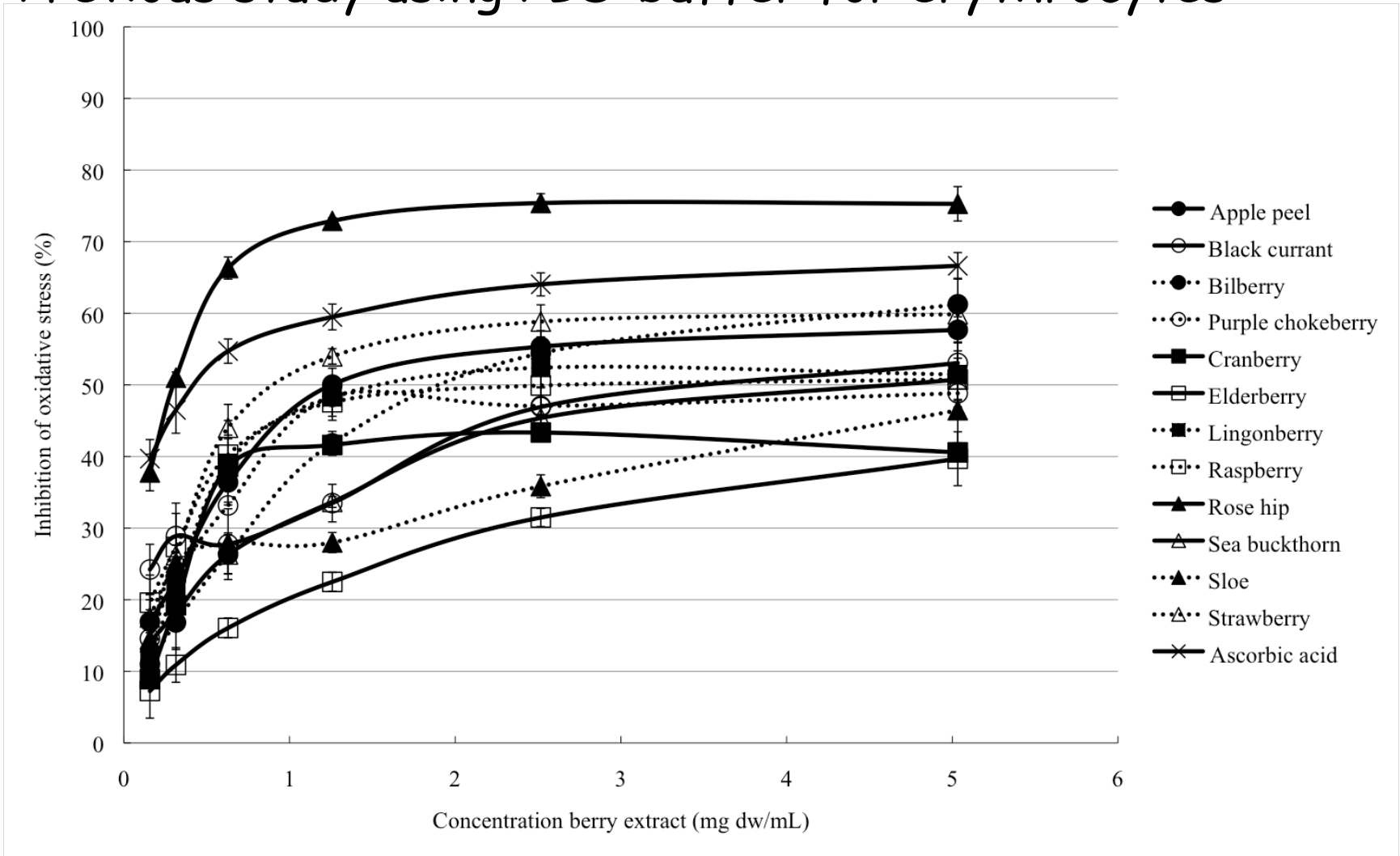
Previous study using PBS-buffer for erythrocytes



Widén et al. 2012c

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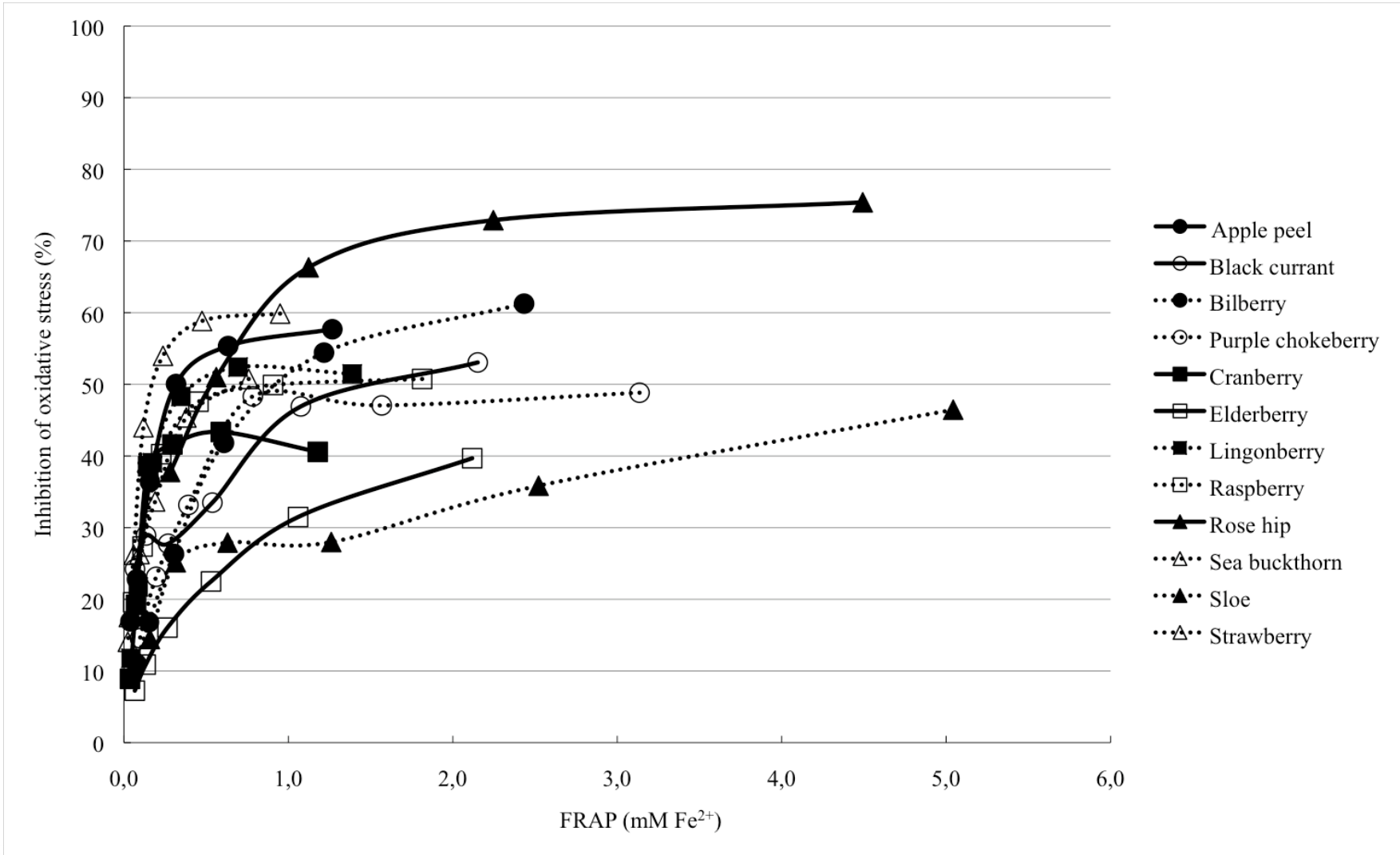
Previous study using PBS-buffer for erythrocytes



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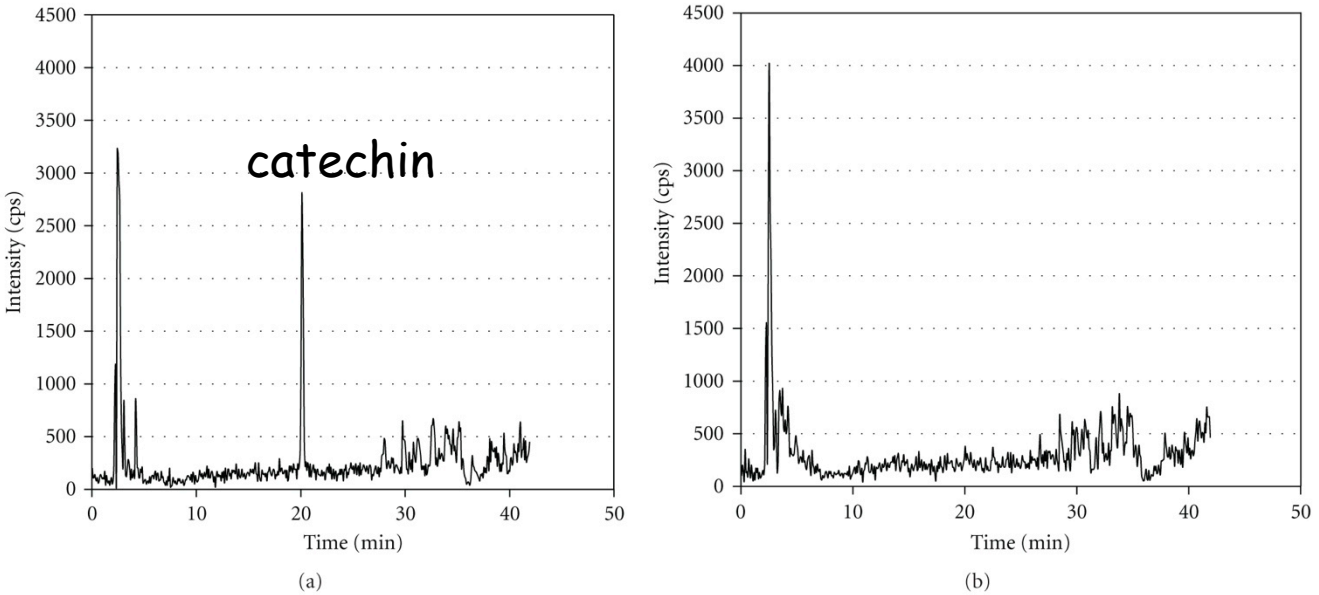
Uptake of polyphenols in erythrocytes treated with a polyphenol-rich rose hip extract: catechin enters the cells!



A) treated erythrocytes B) control

Widén et al. 2012b

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Conclusions and comments

Although there was no significant correlation between the contents of ascorbic acid and protective effects of erythrocyte it was noticed that all samples that contained ascorbic acid protected the cells against oxidation.

High ascorbic acid levels such as in the rose hip extracts which caused a pro-oxidant effect are not present in human blood – and ascorbate is known to be capable of pro-oxidant as well as antioxidant effects.

There is evidence for polyphenol uptake by erythrocytes!

The buffer solution for keeping the erythrocytes has an impact on the results. SagM solution is closer to in vivo conditions than PBS solution. Whilst the clinical relevance of the erythrocyte assay requires further investigation, it remains the more biologically relevant of the assays employed and is likely to provide additional insights into the potential bioefficacy of different berry extracts.

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Further studies

Content of different bioactive compounds in the different extracts

Effects of single berry polyphenols on protection of erythrocytes

Effects of complex mixtures of berry polyphenols on protection of erythrocytes

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Literature

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