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Swedish University of Agricultural Sciences

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Can antioxidant and antibacterial plant extracts make meat products healthier?

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Sustainable plant ingredients for healthier meat products – proof of concepts

Presentation at the 31st EFFoST International Conference, Sitges, Spain 2017

Project consortium

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Project background 1

- A high intake of especially red and processed meat is associated with increased colorectal cancer risk.
- Meat is a healthy food item, having proteins of high biological value, high content of essential minerals and B-vitamins.
- Meat contains pro-oxidative iron but lacks antioxidants.
- Meat products are frequently tainted by microorganisms and therefore nitrite is often added. Nitrite can however form cancerogenic nitrosamines.

Project background 2

- Many plants are rich in natural antioxidants and some plants also contain significant antimicrobial compounds
- Horticultural waste material could also be used for production and extraction of beneficial food ingredients for use in meat products

Project research hypotheses

- 1. Incorporation of complex antioxidant plant extracts in processed meat products will result in healthier products due to decreased level of oxidation in the meat, thus reducing or preventing the inflammation reactions that follows upon consumption of meat products and eventually can result in colon cancer.

- 2. Plants or extracts from horticultural plant materials and its processing side streams can have unique or synergistic antimicrobial effects in different meat products

Project main objectives

1. Collection, preparation, extraction, screening of antioxidative activity/ antimicrobial properties, and characterization of horticultural plant material and side streams

2. In vitro screening of antioxidant effects in meat and meat products using relevant methods, including a model with sarcoplasmic proteins

3. Development of functional food ingredients and pilot plant processing methods

4. Development of conceptual innovative meat products

5. Proofing healthier conceptual meat products by animal studies

Example of plant material sampled

Plant material / waste material being sampled

Aegopodium podagraria (goutweed)
Allium cepa (onion)
Allium ursinum (ramson)
Armoracia rusticana (horseradish)
Aronia x prunifolia (purple chokeberry)
Beta vulgaris (red beet)
Chaenomeles japonica (Japanese quince)
Daucus carrota (carrot)
Fagopyrum esculentum (buckwheat)
Hippophae rhamnoides (sea buckthorn)
Lonicera coerulea var edulis (blue honeysuckle)
Rheum rhaponticum (rhubarb)
Ribes nigrum (black currant)
Ribes rubrum (red currant)
Rosa rugosa (Japanese rose)
Satureja hortensis (summer savory)
Taraxacum officinale (dandelion)
Urtica dioica (nettle)
Vaccinium myrtillus (bilberry)
*Vaccinium vitis-idae*a (lingonberry)




Methods

Extraction

- None, water or 50% ethanol-water
- PHWE

Antioxidative activity (in vitro and in situ)

- DPPH, ABTS radical scavenging
- Inhibition of lipid peroxidation (+ model with sarcoplasmic proteins)
- FRAP
- Total phenols

Antimicrobial activity

- Agar diffusion tests, challenge tests

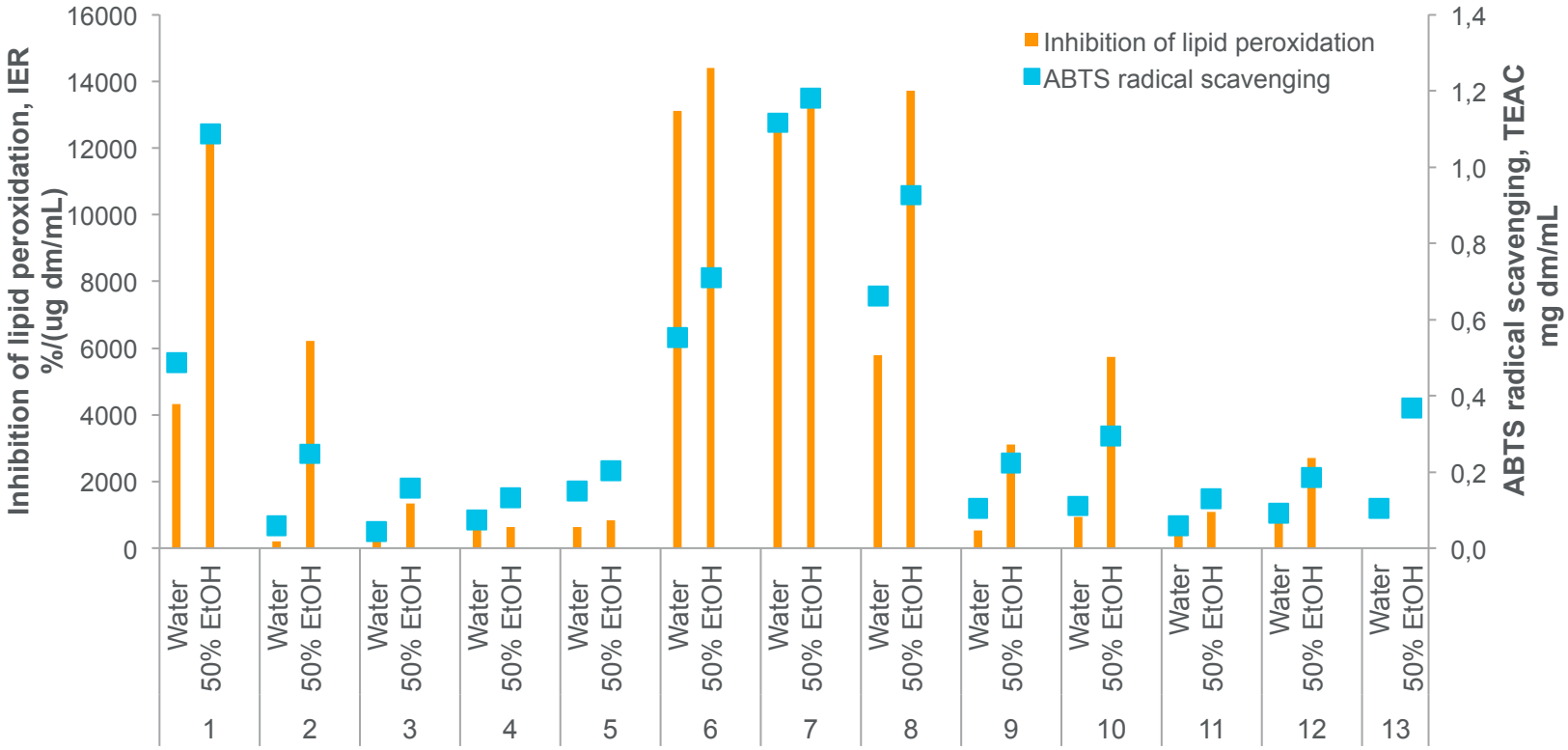
Chemical characterization

- HPLC methods

Sensory acceptability tests

Animal trials

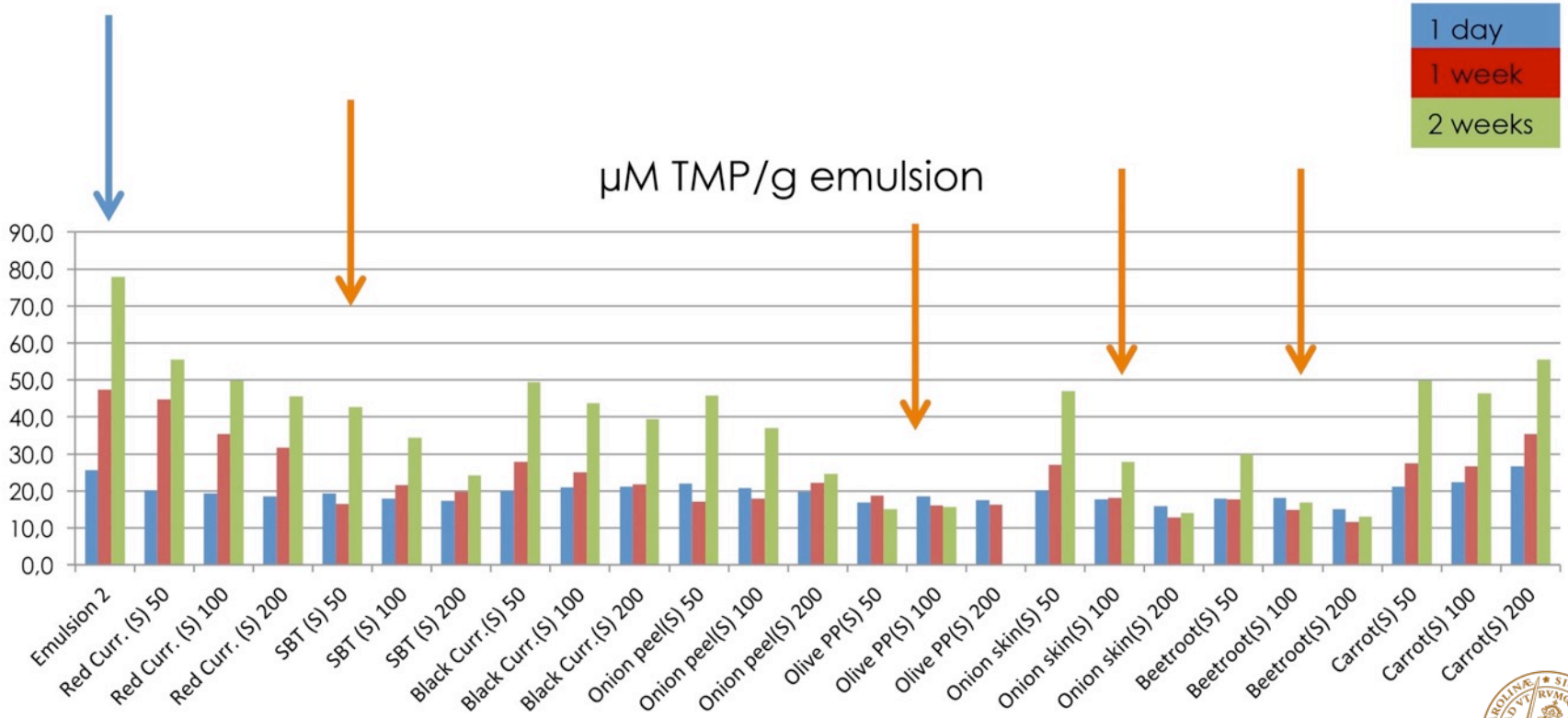
Antioxidant activity in vitro: effect of plant material, solvent and testsystem



1 Sea buckthorn leaf Finland
2 Buckwheat bran
3 Chokeberry
4 Blackcurrant
5 Rose hip
6 Bilberry leaf
7 Lingonberry leaf
8 Sea buckthorn leaf Germany
9 Goutweed
10 Spruce shoot
11 Nettle leaf
12 Dandelion leaf
13 Blackcurrant juice press cake

Courtesy by Mäkinen et al., LUKE

Prevention of lipid oxidation by beetroot, onion, olive and sea buckthorn extracts in vitro (model with sarcoplasmic proteins)



Courtesy by Burri et al., LU and SLU

Concept: multi-component plant antibacterial preservation ingredients as alternative to nitrite

- Exploit possible additive or synergistic effects of different single compounds/single plants.
- Low concentration of single compounds (plant products) – small effect on taste of each component.
- Aim for high antibacterial effect with little effect on taste.
- Focus on *Listeria monocytogenes* in meat – potential to prolong shelf life without nitrite.
- Test plants single or in mix in different concentrations.
- Use in vitro lab model first, followed by 'challenge test' in raw chicken breast meat.
- Aim at a surface applied antibacterial marinade.

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Antibacterial activity: Species tested individually at 1-5 % concentration

- Example of test results at 2.5 and 5 % concentrations in BHI model

Plant species	MIC-value range (percentage)
Summer Savory	2.5 - 3%
Horseradish	3 - 5%
Lingonberry	1.5 - 2%
Red Currant	2 - 5%
Ramson	1.5 - 2%
Rhubarb	2 - 5%

Plant species	Concentration (ppm)	Concentration (Percentage)	*Listeria innocua*; Log cfu/ml after 48 hours
Summer Savory	50000	5%	2.7
Summer Savory	25000	2.5%	4.7
Rhubarb	50000	5%	CI
Rhubarb	25000	2.5%	2.4
Ramson	50000	5%	CI
Ramson	25000	2.5%	CI
Control 1			10
Control 2			11
Horseradish	50000	5%	CI
Lingonberry	50000	5%	CI
Lingonberry	25000	2.5%	CI
Red currant	50000	5%	CI
Red currant	25000	2.5%	CI
Control 1			9.8
Control 2			9.8

Partial inhibitory response

5% **Summer Savory**
2.5% **Summer Savory**
2.5% **Rhubarb**

Bactericidal

5% **Rhubarb**
5% and 2.5% **Ramson**
5% **Horseradish**
5% and 2.5% **Lingonberry**
5% and 2.5% **Red currant**

- Inoculum = 2.3 log cfu/ml
- Independent triplicates and replicates for each independent sample
- CI = complete inhibition

Courtesy by Jensen et al., AU

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Antibacterial activity: 2- and 3-mixture combinations

2-mixture combinations:

Plant product combinations	Total concentration (percentage)	*Listeria innocua*; Log cfu/ml after 48 hours
Rhubarb (1%) and Horseradish (1%)	2%	1.7
Rhubarb (1.5%) and Horseradish (0.5%)	2%	1.7
Rhubarb (0.6%) and Horseradish (1%)	1.6%	2
Control 1		7.48

3-mixture combinations:

Plant product combination	Total concentration (percentage)	*Listeria innocua*; Log cfu/ml after 48 hours
Rhubarb (0.5%) / Horseradish (0.5%) / RC (0.5%)	1.5%	No inhibition
Rhubarb (0.5%) / Horseradish (0.5%) / LB (0.5%)	1.5%	No inhibition
Ramson (0.5%) / SS (0.5%) / LB (0.5%)	1.5%	2.7

Inoculum = 2 log cfu/mL, independent duplicates

Courtesy by Jensen et al., AU

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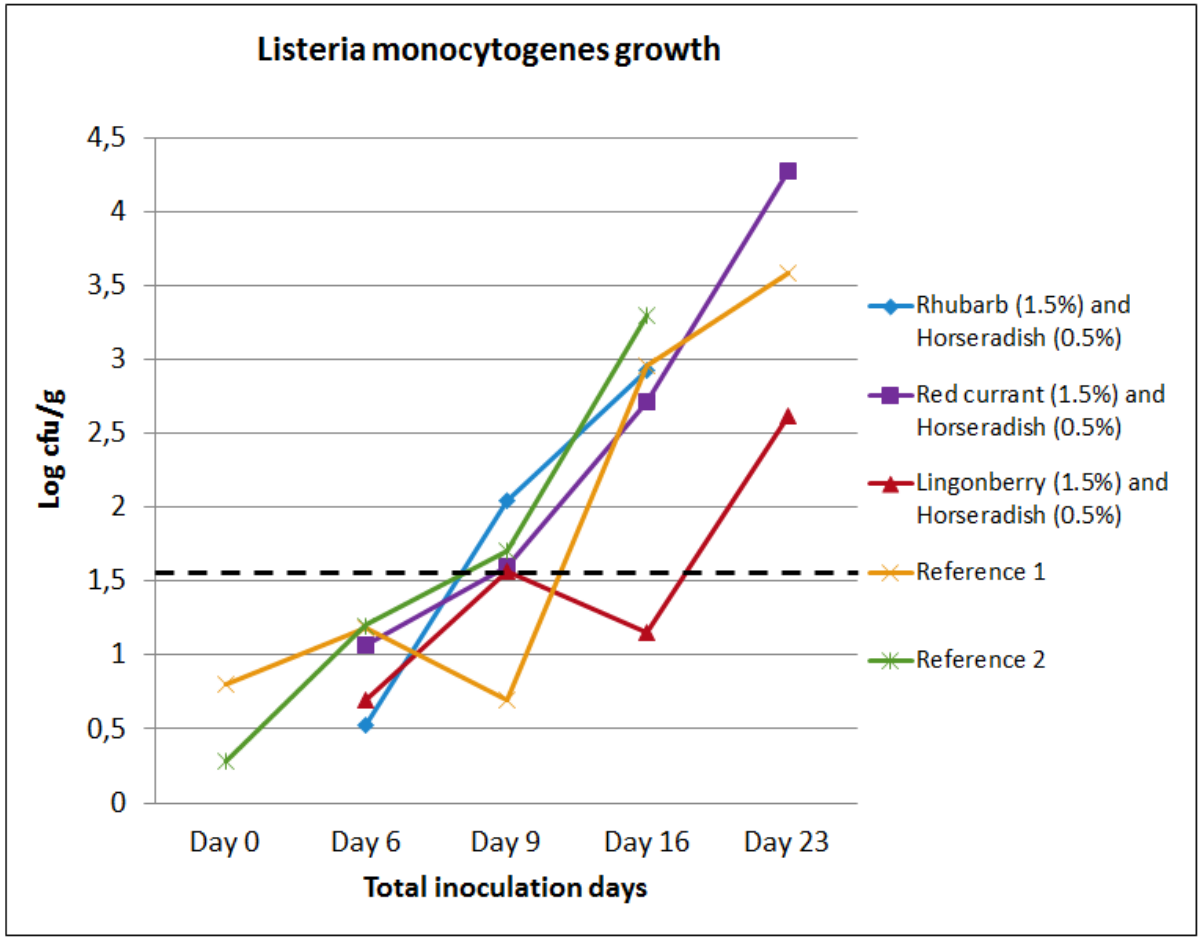
Challenge test - Food safety



Control shelf life:

- around 12 days

Extended shelf life:

- 18-20 days for lingonberry/ horseradish

Inoculum = 1.1 log cfu/g, Independent triplicates, Dotted line is max allowed growth of log 0.5 in challenge test on top of inoculum level

Courtesy by Jensen et al., AU

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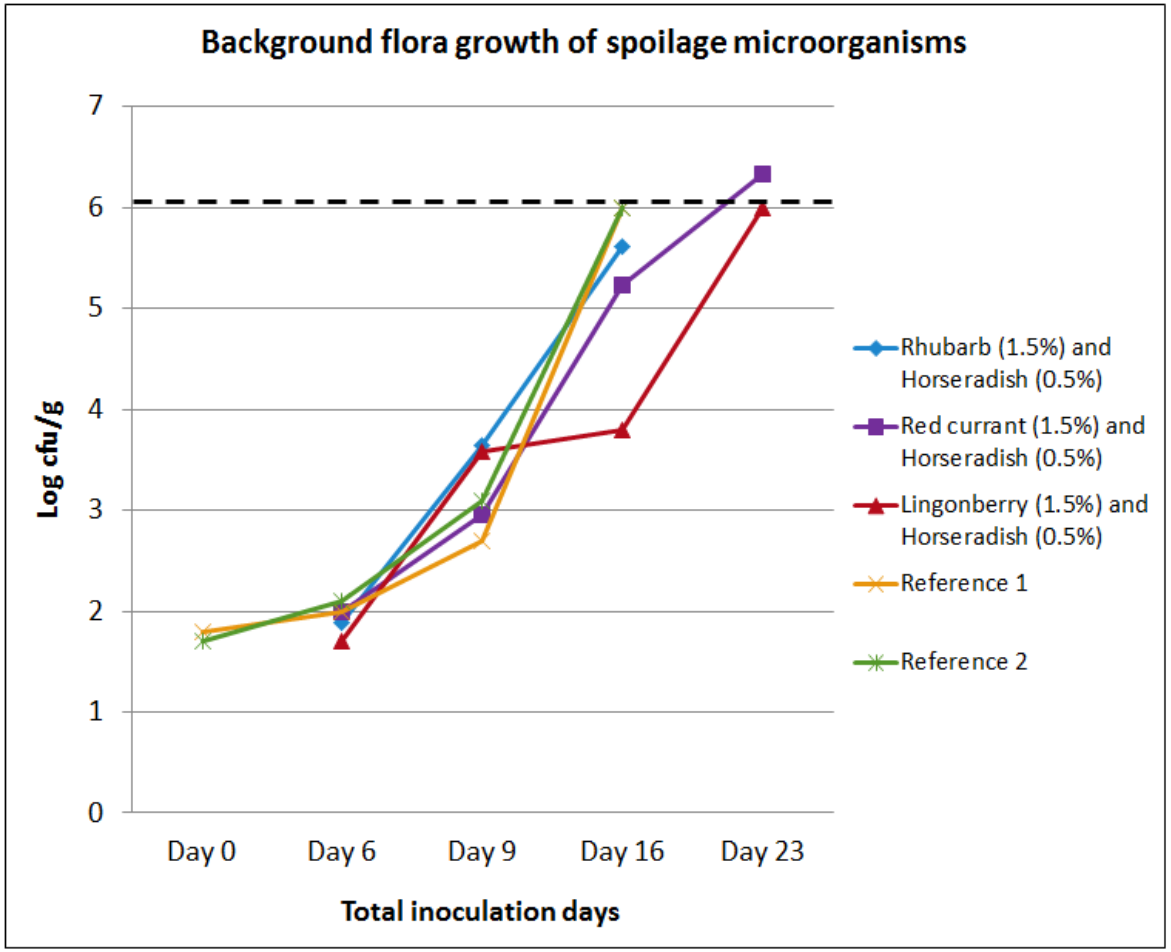
Challenge test – Microbial food spoilage



Control shelf life:

- around 16 days

Extended shelf life:

- 20 days for red currant/ horseradish
- 23 days for lingonberry/ horseradish

Inoculum = 1.1 log cfu/g, Independent triplicates

Courtesy by Jensen et al., AU

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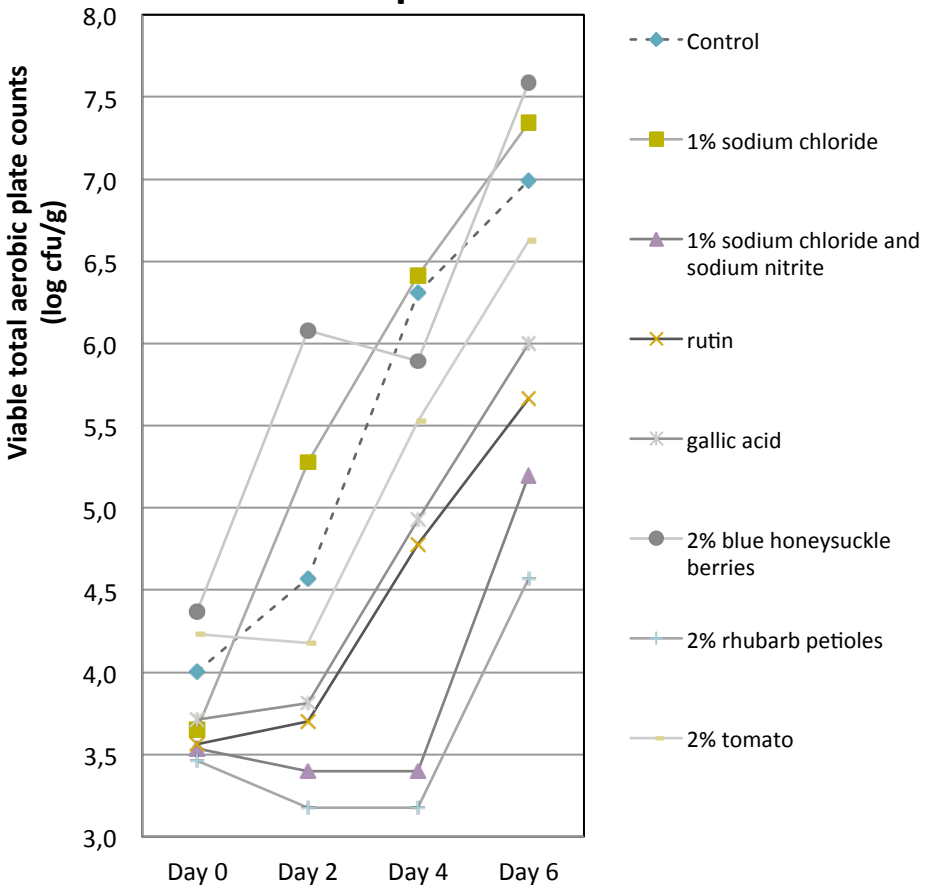
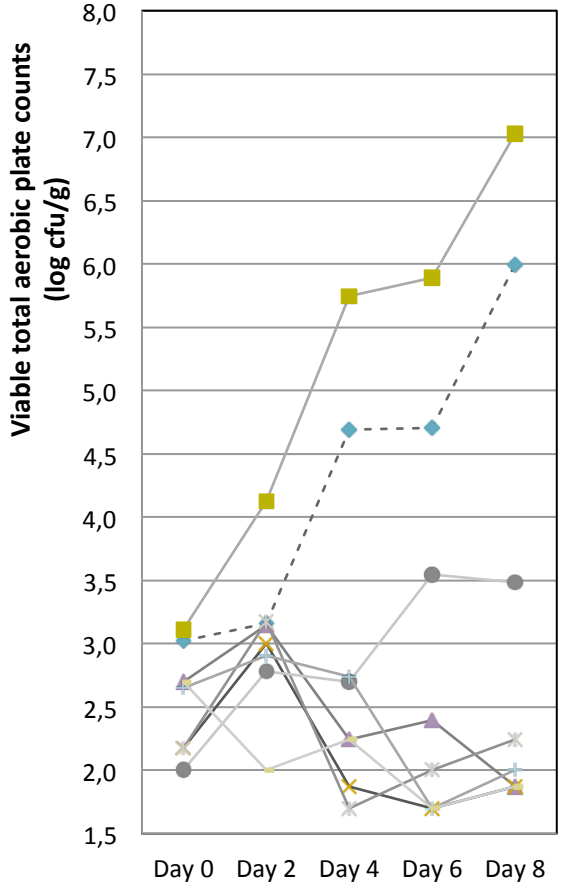
Antibacterial and antioxidative effects in raw and cooked minced pork meat

Raw minced pork meat



Cooked minced pork meat



Conclusion: The most efficient antimicrobials and antioxidants both in raw and cooked minced pork were rhubarb petioles, gallic acid and rutin (and sodium chloride+sodium nitrite).

Concept: pork sausages

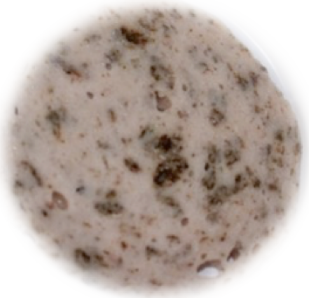
Background

- Sausages were prepared by adding dried bilberry (BBL) or sea buckthorn leaves (SBL) or their extracts (E) to commercial sausage mass of pork prepared without sodium nitrite and ascorbic acid.

SBL

Treatments

- BBL 2 %, SBL1.6 %
- BBLE 0.2 or 1 %, SBLE 0.2 or 1 %



BBL

Results

- At 10 days of storage, BBL 2% concentration and BBLE 0.2 % concentration showed significantly lower lipid oxidation level than the commercial sausage mass
- At 20 days of storage, lipid oxidation in the commercial sausage mass was approx. five fold in comparison to the 10 days storage. For BBL, BBLE and SBLE amended sausages, no increase in lipid oxidation was observed.
- BBL, SBL, BBLE or SBLE did not lower the total aerobic colony counts (PCA, incubation 3 d, 30 ºC) after 24 d of storage at + 6 ºC.

BBLE

Courtesy by Mäkinen et al., LUKE

Concept: marinated sliced chicken legs

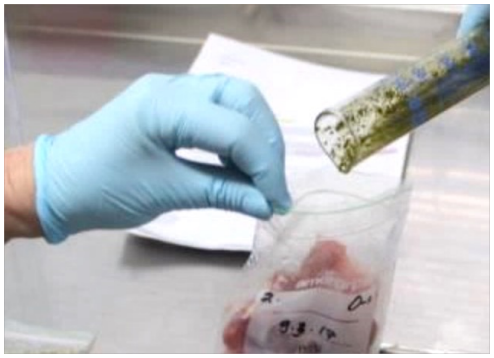
Treatments: Bilberry leaf 4%, sea buckthorn leaf 4%, bilberry leaf extract 0.4% or 2%, sea buckthorn leaf extract 0.4% or 2%, basic marinade (Control) and no marinade.

The test products contained marinade (30%) and chicken slices (70%).
At the beginning pH of marinades was around 3.

Basic marinade	
Rapeseed oil	52%
Sugar	11%
Salt	6%
6% acetic acid	21%

Marinades: 4 % seabuckthorn leaf on the left and bilberry leaf 4 %.

Samples were mixed with marinades and stored in plastic bags at 6 ºC for 4 d.



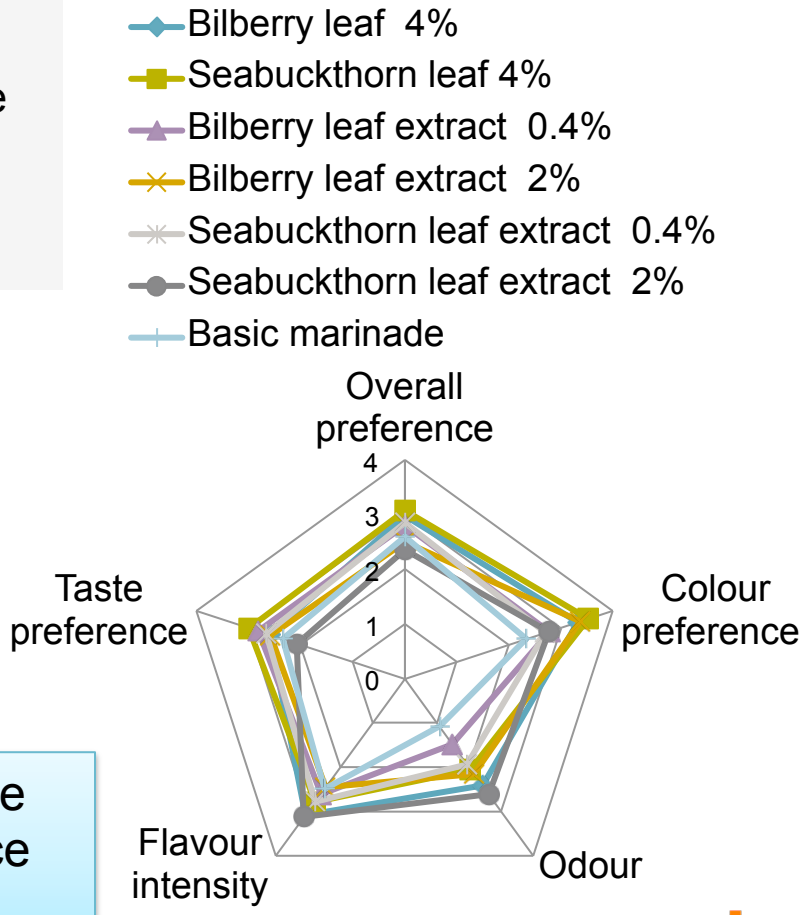
Results: Bilberry leaf, sea buckthorn leaf and PHW extracts of these prevented effectively lipid oxidation. Bilberry leaf at 4% was the most efficient to protect the product from lipid oxidation. No convincing results on microbes during study of storage.

Courtesy by Mäkinen et al., LUKE

Sensory evaluation of meat products

- Sensory evaluation of marinated chicken and sausages was conducted by 10 persons.
- Before sensory evaluation the chickens were fried using ActiFry machine.
- Scales were from 0 (weak) to 5 (strong)

Results of chicken evaluation:



Conclusion: As for both meat products, the strong taste and different visual appearance can be a challenge.

Courtesy by Mäki et al., LUKE

Ongoing: Mice studies

- Colorectal inflammation is induced in mice by cyclic treatment with dextran sodium sulphate (DSS)
- Mice are fed meatballs, with and without antioxidants
- Analyses (living): Weight, disease index/ health assessment
- Analyses (post mortem): Histology, FACS (flow cytometry), Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Analysis of bacterial flora), possibly also antioxidants in porta blood and/or lumen
- Trial has started - results will be available spring 2018

Conclusions

- PHWE is a promising, solvent-free method to obtain bioactive antioxidant and antimicrobial extracts

- Bilberry leaves, sea buckthorn leaves, rhubarb petioles, red beetroot leaves, onion skin, olive oil waste water powder and summer savory were selected as the most potential raw materials based on their antioxidative and/or antimicrobial properties

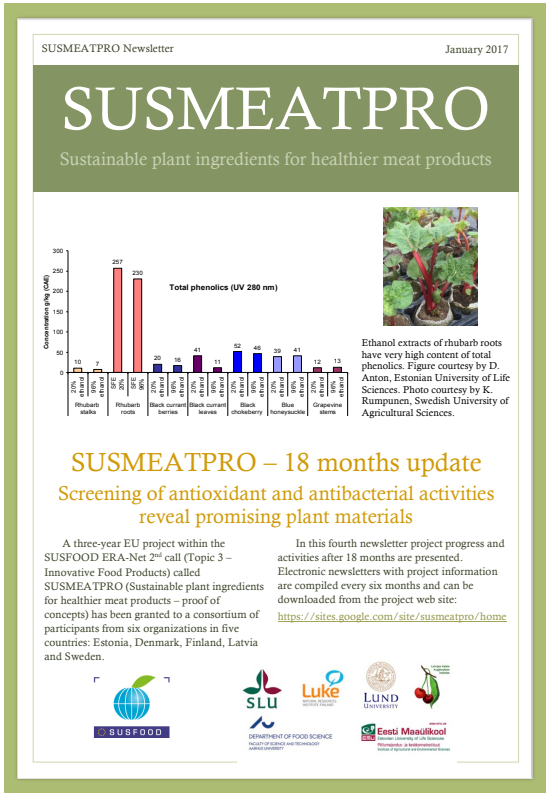
- Antioxidative and antimicrobial effects of plant material and extracts have been documented in conceptual products (sausages, chicken meat, minced pork meat, marinades).

- For antimicrobial activity a combination of plant material make it possible to have an satisfactory effect at lower concentration (eg. lingongberry and horseradish).

- The strong taste and different visual appearance in the products can be a challenge for consumer acceptance using plant extracts.

Acknowledgement

- All partners sharing information and pictures
- All funding agencies
- All companies providing access to plant material and side streams for sampling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Sustainable plant ingredients for healthier meat products

Total phenolics (UV 280 nm)

Ethanol extracts of rhubarb roots have very high content of total phenolics. Figure courtesy by D. Anton, Estonian University of Life Sciences. Photo courtesy by K. Rumpunen, Swedish University of Agricultural Sciences.

SUSMEATPRO – 18 months update

Screening of antioxidant and antibacterial activities reveal promising plant materials

A three-year EU project within the SUSFOOD ERA-Net 2nd call (Topic 3 – Innovative Food Products) called SUSMEATPRO (Sustainable plant ingredients for healthier meat products – proof of concepts) has been granted to a consortium of participants from six organizations in five countries: Estonia, Denmark, Finland, Latvia and Sweden.

In this fourth newsletter project progress and activities after 18 months are presented. Electronic newsletters with project information are compiled every six months and can be downloaded from the project web site: https://sites.google.com/site/susmeatpro/home

Project information

- Home page with newsletters: https://sites.google.com/site/susmeatpro/home