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# Antibacterial effect of herbs and berries against Salmonella, Listeria and E. coli *Berrymeat*

Development of production of organic herbs and berries in Denmark with high and stable preserving effect and unique new taste used in organic meat products (EU aims to reduce nitrite and salt in food)

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## DMRI –Teknol. Inst. (leader)

- Screening plant products for antibacterial effect on Listeria monocytogenes, Salmonella typhimurium, E. coli.
- Single or combined products
- - Radial diffusion assay
- - Bouillon assay
- - Meat sausage products
- Stability – safety, quality
- Sensoric evaluation

## DFS AU – Food science

- Deliver candidate species, cultivars, products
- Formulation and up-concentration of products
- Demonstrate organic cultivation of species
- Impact of cultivars, harvest time, processing, storage on preservation effect in selected sp.
- Content of bioactive compounds – correlation to preservation effect.
- Ensure reproducibility – safe foods and correct dosis

MAPP, AU

Consumer aspects

Focus groups – acceptance, species, methods, taste

Final products – attitudes, preferences

Meat companies

Tulip – conventional

Hanegal - organic

Applied large scale testing

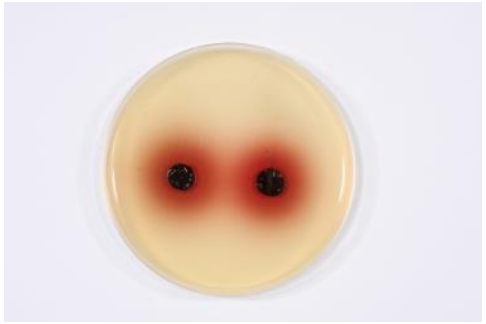
- **Choosing species /cultivars after their content of antibacterial compounds**

- Acids – malic acid, ascorbic acid, citric acid (pH)
- Phenolics – flavonoids, anthocyanins, tannins
- Terpenes – essential oils
- Isothiocyanate
- Alicin, Aliin

- **Challenges**
- Combining different species with different compounds
  - additive or synergistic preservation effect
  - reduce problems of inferior taste due to high concentration of one species/compound

- Formulation – powder/slurry/pesto – homogenous distribution
- Pasteurisation needed of herbs and berries - effect on bioactive compounds?
- Up – concentration needed? Extraction?
- Water soluble or oil soluble? Delivering preservative effect in water phase in meat.

- Combining taste - preferences



- Focus : Listeria, Salmonella and E. coli.
- Identification of candidate plant species.

- **1. Pre-screening** in Radial diffusion assay.
- **2. Bouillon screening test** (36 species).
- **3. Meat slurry solution test** (8 species)
- 4. Effect of **processing** for increased preservation effect.
- 5. Effect of **storage on** preservation effect.
- **6. Examine content of bioactive compounds depending on** 4 and 5 in few sp.

- **5. Organic cultivation trials in** 3-5 chosen species:
  Effect of **cultivars**, clonal differences
  Effect of **harvest time, year**
- Effect of organic cultivation methods (**fertilizer**)

- Impact on **preservation capacity and concentration of bioactive compounds** (correlations and understading of preservation effect)

- **Aim: optimal quality**, safe dosis, **reproducibility o**f preservation effect.

Results from screening in Radial Diffusions Assay (RDA) and bouillonmodel.

| ***Species*** | | ***Radial diffusions Assay*** | | ***Bouillon model 3% salt and 10 % added*** | |
|---|---|---|---|---|---|
| **Popular name** | **Product** | ***Listeria*** | ***Coli/Salmonella*** | ***Listeria*** | ***Coli/Salmonella*** |
| Aronia (sortrøn (surbær) | Berry,<br>juice<br>pomace | No<br>3 cvs: **good effect**<br>nd | No<br>No<br>No | | |
| **Aronia "Nero" Aronia"Viking"** | **Mash** | **effect** | **effect** | **No effect** | **No effect** |
| Vaccinium corymbosum | Berries<br>Juice | No<br>No | **Good effect**<br>No | | |
| Vaccinium myrtillus | Berries<br>Juice | No<br>No | **Good effect**<br>No | | |
| Vacinium ulignosum | Berry mash | **Some effect** | **Some effect** | No effect | No effect |
| **Vaccinium vitis ideae** | Berry mash | **Effect** | **Effect** | **Effect** | **Effect** |
| **Vaccinium vitis ideae cv. Red Pearl** | Berry mash | **Effect** | **Effect** | **Effect** | **Effect** |
| **Vaccinium macrocarpon Stevens** | **Berry mash** | **Effect** | **Effect** | **Effect** | **Effect** |
| **Ribes nigrum (7 cvs)** | Berries<br>Juice | **Good effect**<br>**No** | **Good effect**<br>**Good effect** | **Effect** | **Effect (no diff. cvs)** |
| **Ribes rubrum (7 cvs)** | Berry mash | **Good effect** | **Good effect** | **Effect** | **Effect (diff. cvs)** |
| **Rubus idaeus Erika** | **Berry mash** | **Effect** | **Effect** | **Effect** | **Effect** |
| Rubus fruticosus cv. "Loch Tay" | **Berry mash** | No effect | No effect | No effect | No effect |
| **Hippophae rhamnoides AU** | **Berry mash** | **Good effect** | **Small effect** | **No effect** | **Effect** |
| **Hippophae rhamnoides** | **Powder Eskærhus** | **Effect** | | **Effect** | **Effect** |
| Rosa rugosa | **Berry mash** | No effect | No effect | No effect | No effect |
| Rosa canina | **Berry mash** | No effect | **Some effect** | No effect | No effect |
| **Sambucus nigra** | **Berry mash** | **No effect** | **Some effect** | **No effect** | **No effect** |
| **Sambucus nigra** | **Dried berry** | **Effect** | **Effect** | **Effect** | **Effect** |
| **Sambucus nigra** | Flower | No effect | **Some effect** | No effect | Small effect |

| **Species** | | **Radial diffusions Assay** | | **Bouillon model 3% salt and 10 % addition** | |
|---|---|---|---|---|---|
| **Popular name** | **Product** | ***Listeria*** | ***Coli/Salmonella*** | ***Listeria*** | ***Coli/Salmonella*** |
| **Prunus spinosa** | **Berry mash** | **Effect** | **Effect** | **Effect** | **No effect** |
| Malus sylvestris | Fruit mash | No effect | No effect | No effect | No effect |
| Capsicum Chili (5 cvs) | Fruit | No effect | No effect | No effect | No effect |
| Carum carvi | Seed+ EtOH | No effect | No effect | | |
| Juniperus communis | Berry mix green and black | No effect | No effect | **Small effect** | No effect |
| Juniperus communis | Needles | No effect | No effect | **Small effect** | No effect |
| Empetrum | Berry mash | No effect | No effect | No effect | No effect |
| Origanum vulgare subsp. Hirtum | Leaves (H2O) | **Some effect** | No effect | No effect | No effect |
| Origanum vulgare | Leaves | No effect | No effect | No effect | No effect |
| **Thymus vulgaris** | Leaves (H2O) | No effect | No effect | **Effect** | No effect |
| Rosmarinus officinalis | Leaves (H2O) | No effect | No effect | No effect | No effect |
| **Salvia officinalis, cv. Phasa** | Leaves (H2O) | No effect | No effect | **Effect** | No effect |
| **Satureja montana** | Leaves (H2O) | **Some effect** | No effect | **Effect** | No effect |
| Satureja hortensis | Leaves (H2O) | No effect | No effect | No effect | No effect |
| Mentha x piperata cv Peppermint | Leaves (H2O) | No effect | No effect | No effect | No effect |
| Mentha green mint | Leaves (H2O) | No effect | No effect | No effect | No effect |
| Matricaria recutita | Leaves (H2O) | No effect | No effect | No effect | No effect |
| **Myrica gale** | **Leaves male** | **Effect** | No effect | **Effect** | No effect |
| **Myrica gale** | **Leaves female** | **Effect** | No effect | **Effect** | No effect |
| **Myrica gale** | **Fruit/seed female** | **Effect** | No effect | **Effect** | No effect |
| **Humulus lupulis (6 cvs)** | **Flower** | **Good effect** | **No effect** | **Effect (no diff. cvs)** | **No effect** |
| **Rheum rabarbarum (5 cvs)** | **Stalk** | **Good effect** | **Some effect** | | |

| | | | | | |
|---|---|---|---|---|---|
| ***Allium sativa (2 cvs)*** | ***Onion*** | ***Super effect*** | ***Super effect*** | ***Good effect*** | ***Good effect*** |
| **Ramsons** | **Onion** | **Good effect** | **Good effect** | **Good effect** | **Good effect** |
| **Armoracia rusticana (5 cvs)** | **Root** | **3 cvs small effect** | **No effect** | **No effect** | **No effect** |

**1. – 2. screening**

Aronia melanocarpa
Vaccinium corymbosum
Vaccinium myrtillus
Vaccinium ulignosum
Vaccinium vitis-ideae
Vaccinium macrocarpon
Ribes nigrum
Ribes rubrum
Rubus idaeus
Rubus fruticosus
Hippophae ramnoides
Rosa rugosa
Rosa canina
Sambucus nigra
Prunus spinosa
Malus sylvestris
Capsicum annuum
Carum carvi
Juniperus communis
Origanum vulgare subsp. Hirtum
Origanum vulgare
Thymus vulgaris
Rosmarinus officinalis
Salvie officinalis
Satureja montana
Satureja hortensis
Mentha x piperatae
Matricaria recutita
Myrica gale
Humulus lupulis
Rheum rhababarum
Allium sativa
Allium ursinum
Armoracia rusticana

**Species examples with some/good effect on LM and/or S-E) (about 20 in total with fair effect)**

Aronia melanocarpa(Black chokeberry, sortrøn)
Vaccinium myrtillus (Blueberry, blåbær)
Vaccinium macrocarpon (cranberry)
Vaccinium vitis ideae (lingon berry)
Ribes nigrum and rubrum (Black and red currant)
Rubus idaeus (raspberrry)
Hippophae rhamnoides (Sea buckthorn)
Sambucus nigra (Elderberry)
Prunus spinosa (Sloe, Slåen)
Humulus lupulus (Hop flowers)
Rheum rhabarbarum (rhubarb)
Salvia officinalis (Common sage)
Satureja montana (Winter savory)
Myrica gale (porse)
Allium ursinum (Ramsons, wood garlic)
Armoracia rusticana (Horse radish)






# 8 model species for further tests

**(Selection criteria: inhibition, all or one bacteria, taste, representing organ)**

Aronia melanocarpa(Black chokeberry)
Vaccinium vitis ideae (Lingon berry)
Prunus spinosa (Sloe)
Ribis rubrum (red currant)
Salvia officinalis (common sage)
Satureja hortensis (Sommer savory)
Allium ursinum (Ramsons, wood garlic)
Armoracia rusticana (Horse radish)

8 species

4 formulations (fine, homogenised)

Effect on bacteria inhibition in 'meat slurry test'.

1. Freeze dried, powder (Frozen)
2. Owen dried 50°C, powder (Frozen)
3. Fresh, non-dried, macerated mash/pesto(Frozen)
4. Fresh, non-dried, macerated, pasteurised, 92°C 10 min. (Frozen)

- Storage for 12 months, stability of antibacterial effect.
- Measure content of main antibacterial compopunds

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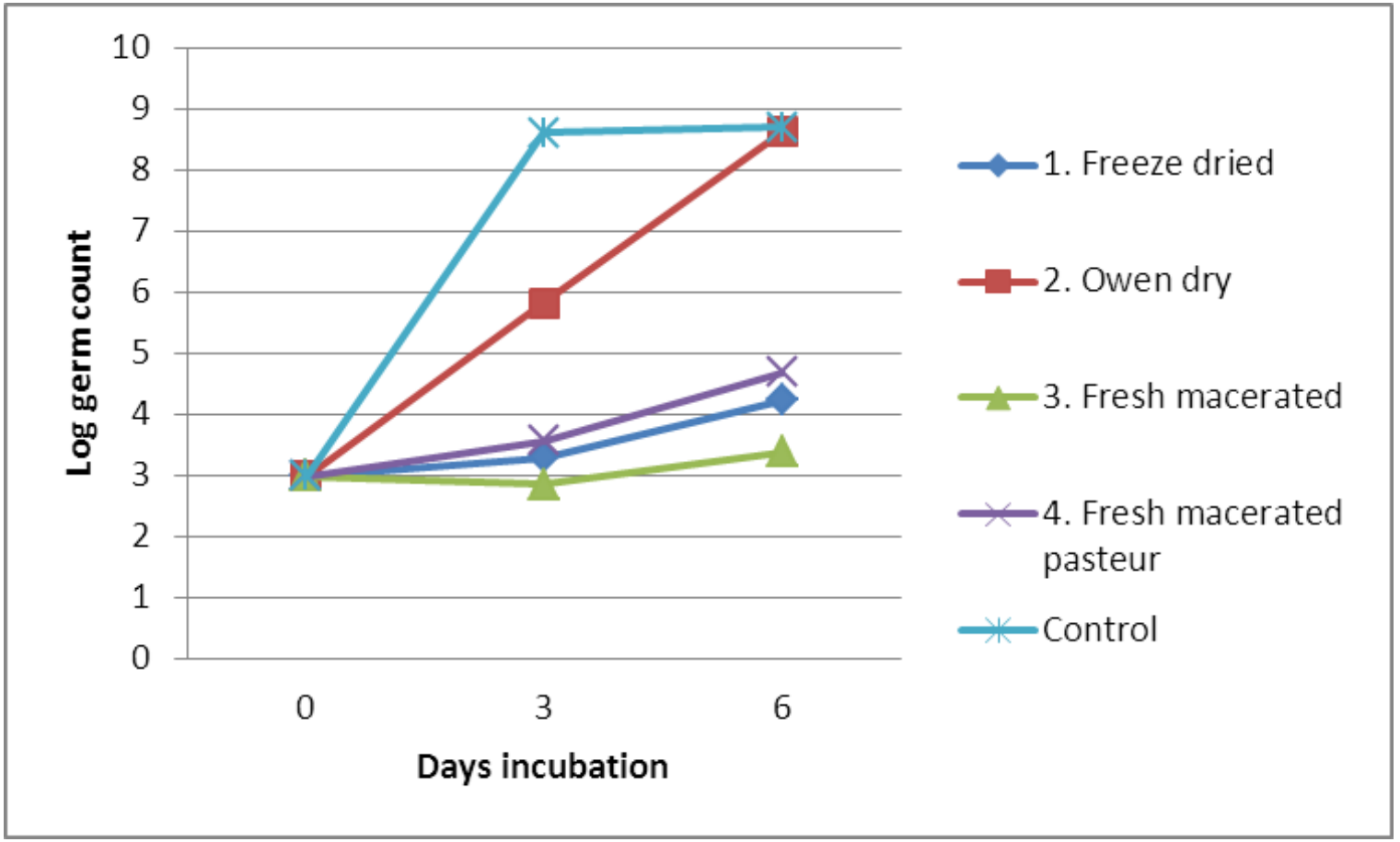
# Red currant Rovada

# Horse radish
# Sindal

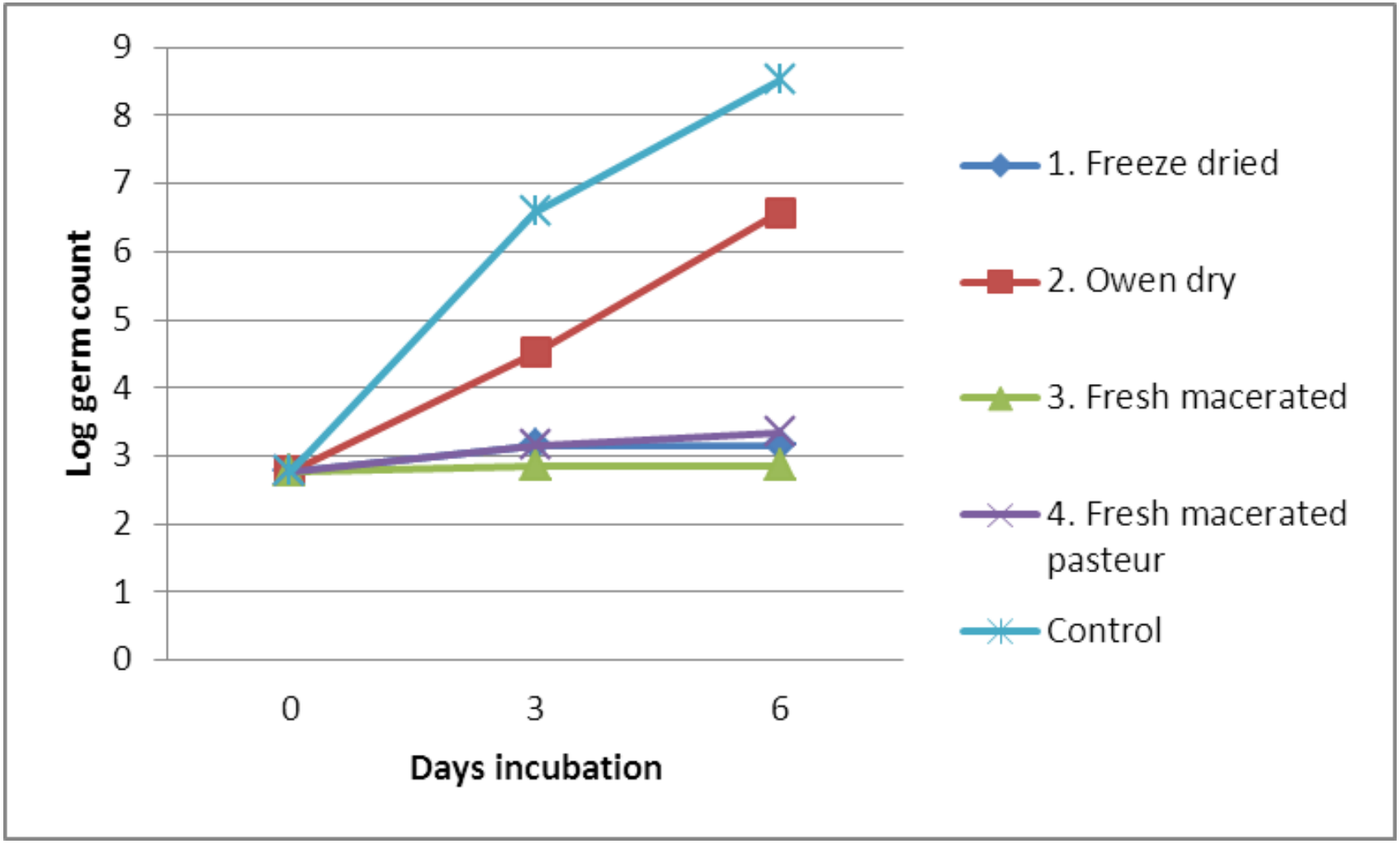


| | Freeze dried | Owen dried 50 °C |
|---|---|---|
| Aronia |  |  |
| Horse radish |  |  |
| Ramsons |  | |
| Red currant |  |  |

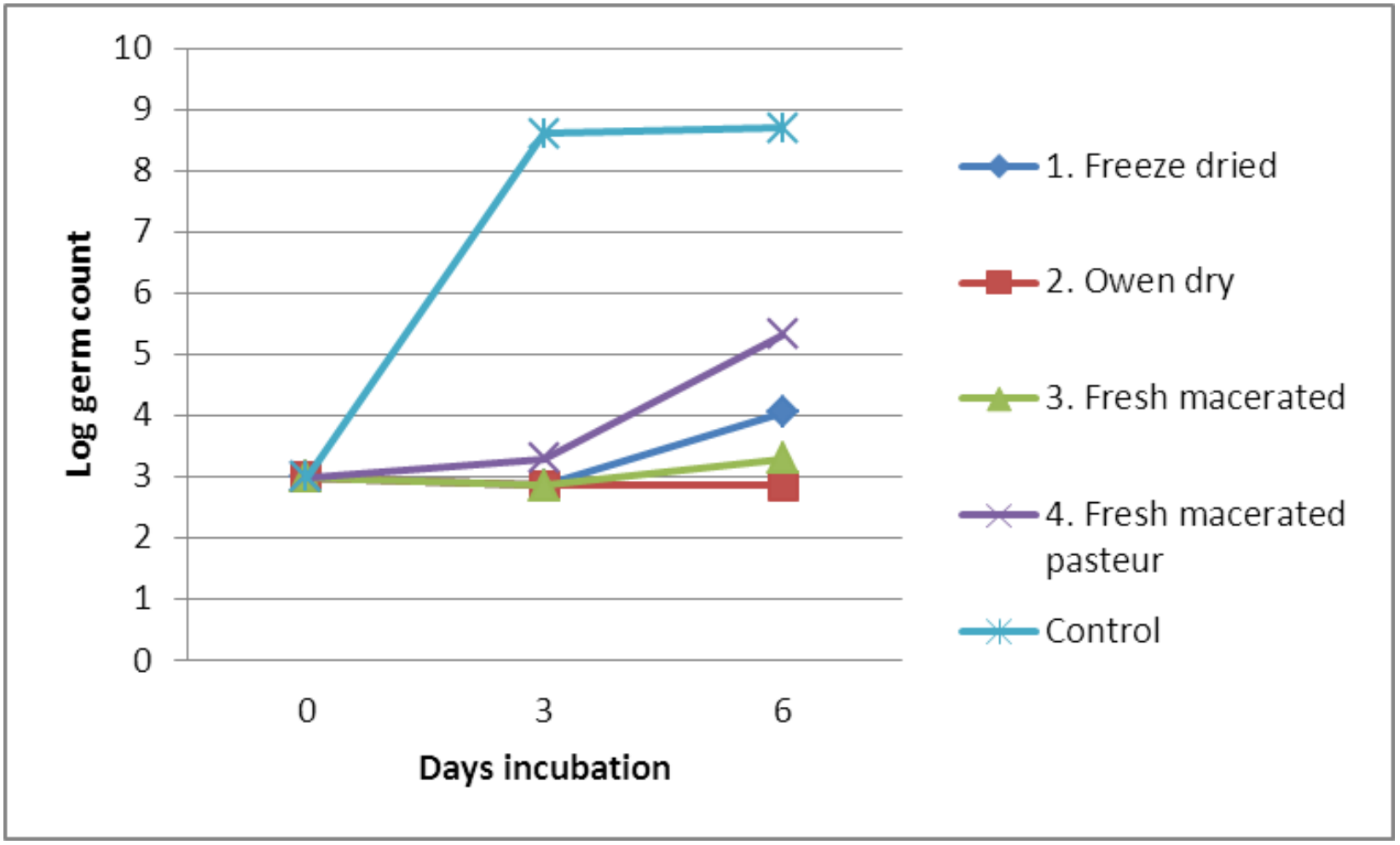
| | Freeze dried | Owen dried 50 °C |
|---|---|---|
| Sage |  |  |
| Sloe |  |  |
| Savory |  | |
| Lingon berry |  |  |



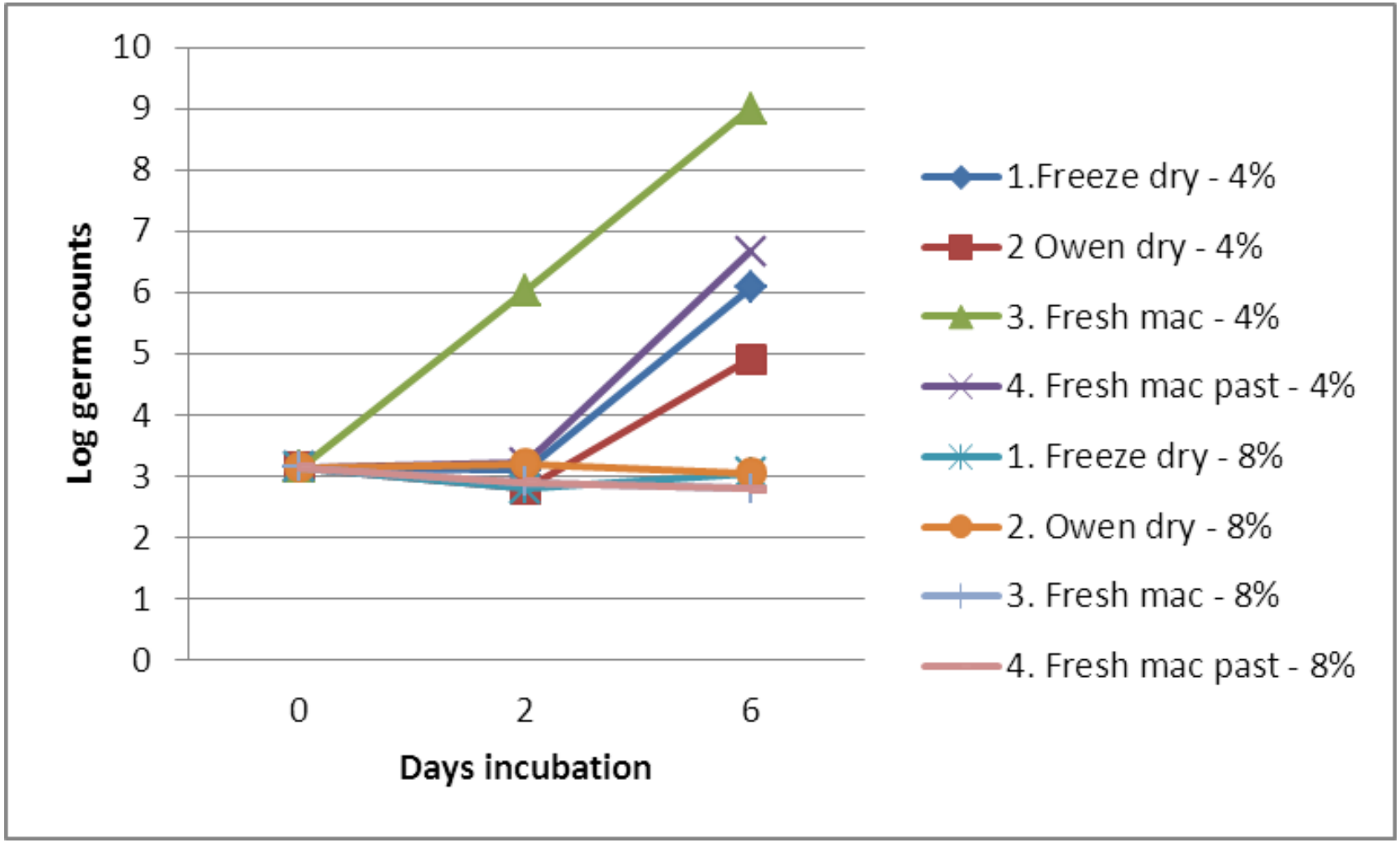

Effect of 4 formulations of **lingon berry** added to a meat slurry test on inhibition of **Listeria** (8 % added - by dry weight*).




Effect of 4 formulations of **lingon berry** added to a meat slurry test on inhibition of **Salmonella** (8 % added by dry weight*).




Effect of 4 formulations of **Sage** added to a meat slurry test on inhibition of **Listeria** (8 % added by dry weight).

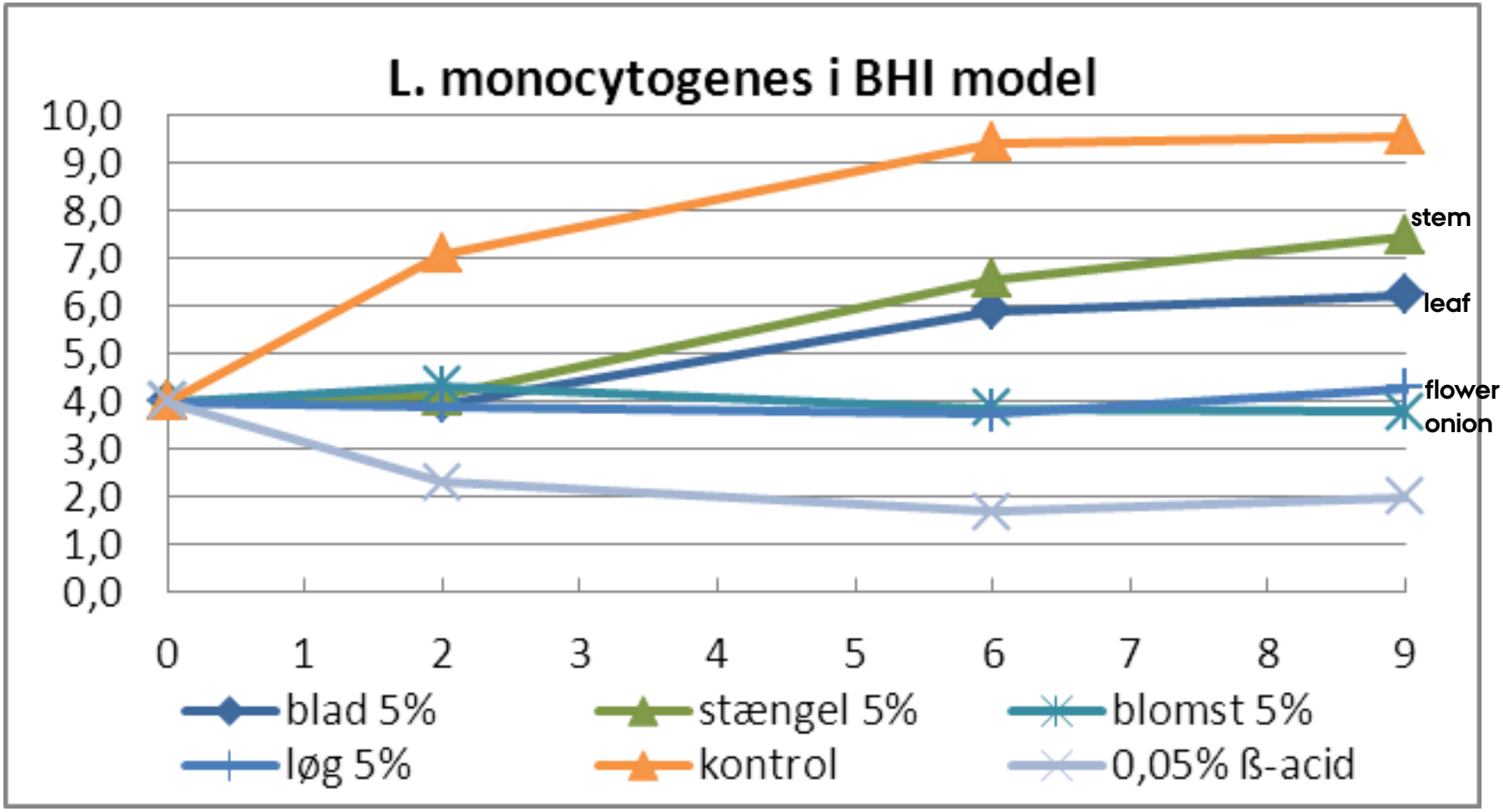



Effect of 4 formulations of **horse radish** added to a meat slurry for testing inhibition of **Listeria** (4 or 8 % added).

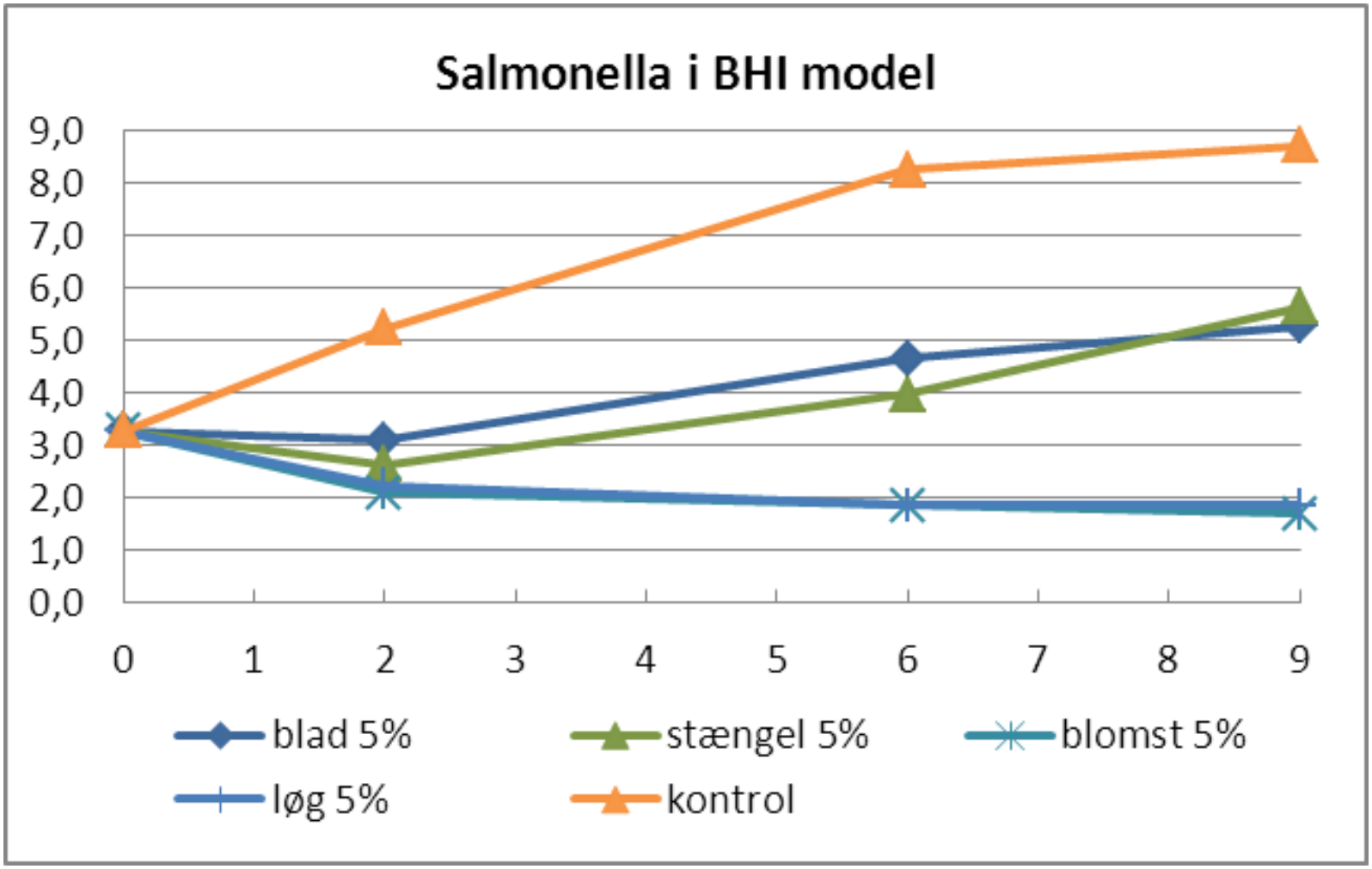
Inhibition of bacteria by plant species (8 % concentration).

| | Listeria | Salmonella | E. coli |
|---|---|---|---|
| Aronia | | | |
| Lingon berry | X | X | X |
| Red currant | X | X | X |
| Sloe | | | |
| Ramsons | X | X | X |
| Savory | X | | |
| Sage | X | | |
| Horse radish | X | X | X |

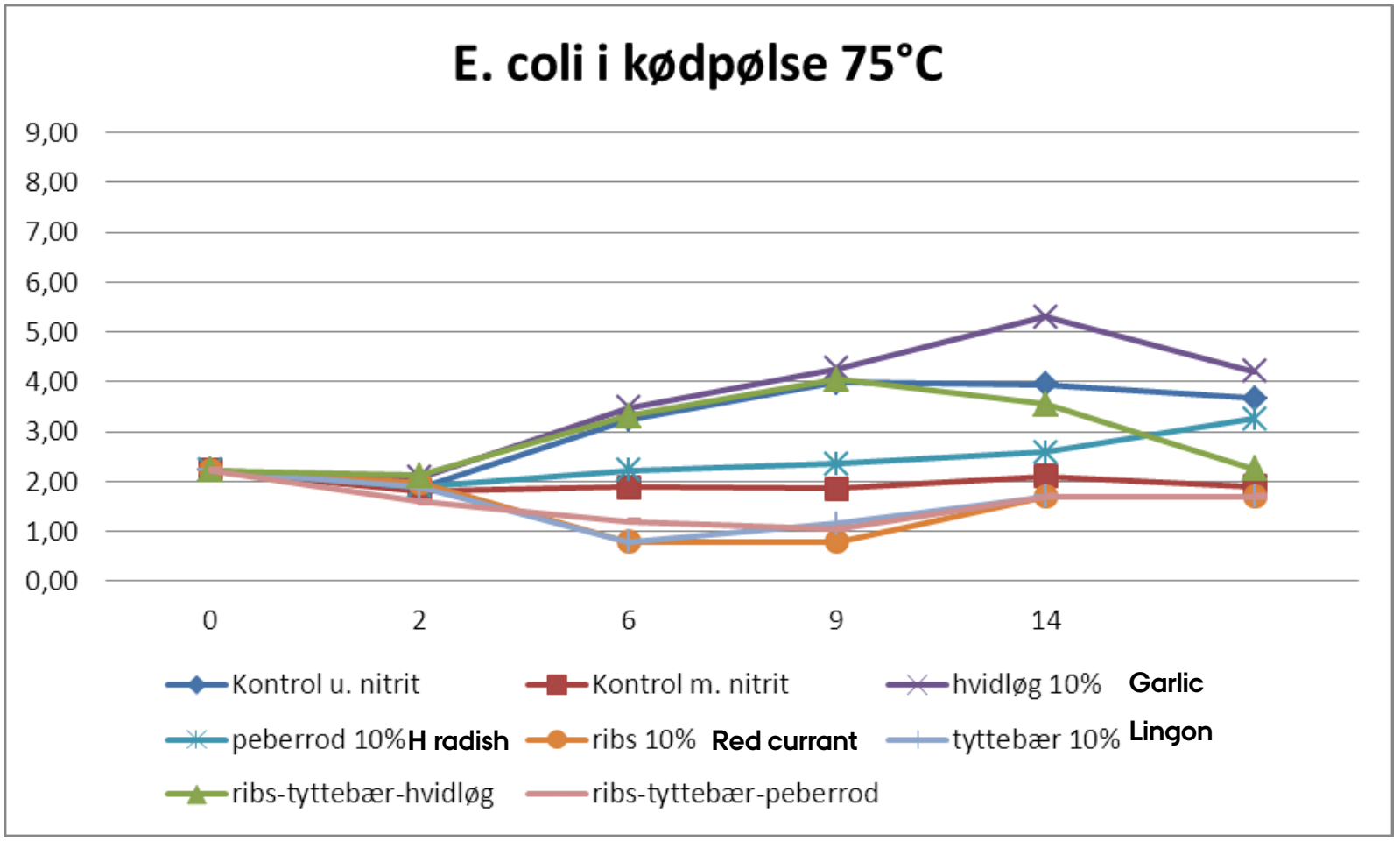
Red currant and lingon berry lowers pH in meat slurry to about 5, and may cause inhibition due to pH alone.




**Ramsons**: Comparison of flower (blomst), leaf (blad), stalk (stængel) and onion (løg) on inhibition of **Listeria** bacteria in meat bouillon model, 5 % addition

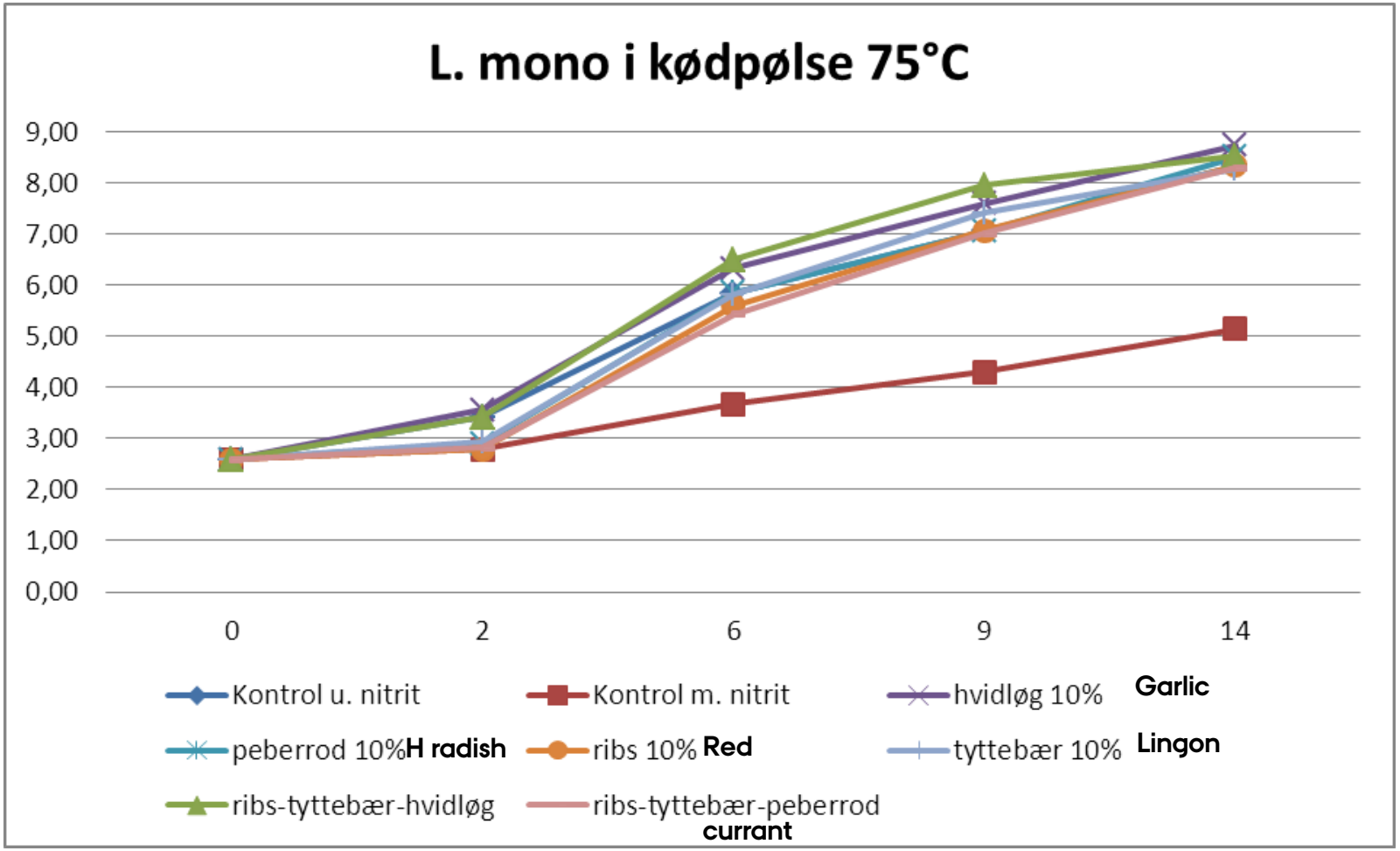



**Ramsons**: Comparison of flower (blomst), leaf (blad), stalk (stængel) and onion (løg) on inhibition of **Salmonella** bacteria in meat bouillon model, 5 % addition [21]




Growth of **E. coli** in meat sausage with 10 % addition of herbs/berries or mix of 4+4+4 % during 18 days test.

**Garlic, horse radish, red currant, lingon berry, combinations**




Growth of **Listeria** in meat sausage with 10 % addition of herbs/berries or mix of 4+4+4 % during 18 days test.

**Garlic, horse radish, red currant, lingon berry, combinations**

# Conclusions

- Identified more than 20 species with some antibacterial activity against Listeria, Salmonella, E.coli.
- Difference in activity of formulations – owen drying and pasteurisation may decrease activity
- Depend on concentration, 8-10 % better than 4 %.
- 'Stacking' of bioactivity indicated to be possible
- Little or no effect in meat sausages with high fat content (lipid soluble compounds)
- Further tests on low fat products, increased concentrations
- Need for understanding kinetics/mechanism of inhibition.