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Determination of the microbial profile during the fermentation process of grape leaves brine”

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Fermented foods

- Fermented foods often consist of a diverse group of microorganisms

Fermented foods

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Fermented foods

- Fermented foods often consist of a diverse group of microorganisms → *consortia*

A group of interacting and cooperating microorganisms

(De Filippis et al., 2017)

Fermented foods

- Fermented foods often consist of a diverse group of microorganisms.
- The microbial consortia generating the final fermented products range from simple to very complex harboring lactic acid bacteria, other bacteria, yeasts and molds, and varying in abundance and diversity during the processing.

Fermented foods

- Understanding the diversity of microbiota and the complex interactions between them would allow to modulate them to produce better quality products.

Grapes and viticulture in Turkey

- Turkey ranks 6th in the world grape export (2017-6.3 %)
- Grape is utilized in a variety of products in Turkey
  - Fresh consumption
  - Dried grape
  - Fruit juice
  - Wine
  - Rakı
  - Vinegar
  - Molasses (Pekmez)
  - Pestil
  - Grape leaves

(https://oec.world/en/profile/hs92/0806)

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Grape leaves

- Sarma made from grape leaves is a traditional dish that has been consumed for at least 400 years since Ottoman times.
- In addition to Turkey, popular in Greece, Armenia, Iran, Arabic countries and in the Balkan peninsula

Rich in

- Minerals
- Vitamin C
- Phenolic compounds

(Dogan et al., 2015; Cangi and Yagci, 2017)

Grape leaves – Preservation processes

- Brining
- Canning
- Freezing
- MAP with passive modification

(Cangi and Yagci, 2017)

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- Process conditions vary among regions/processing plants:
- Brine salt concentration
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⇨ Changes in color, flavor and texture

(microbial profile)

Grape leaves – Preservation processes

- Brining ⇨ oldest
  the most widely used technique
- Very few studies
  - Mainly focusing on physical and chemical properties
  - General microbiological properties (microbial counts)

Aim of our study

- to analyze the effect of different salt concentrations on the microbial profile during brining of grape leaves:

- 5% SC (w/v)
- 12% SC
- 19% SC

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- Microbial profile: LAB and yeasts
- Basic chemical properties of brine:
  - Salt concentration
  - pH and titratable acidity
- Basic microbiological properties

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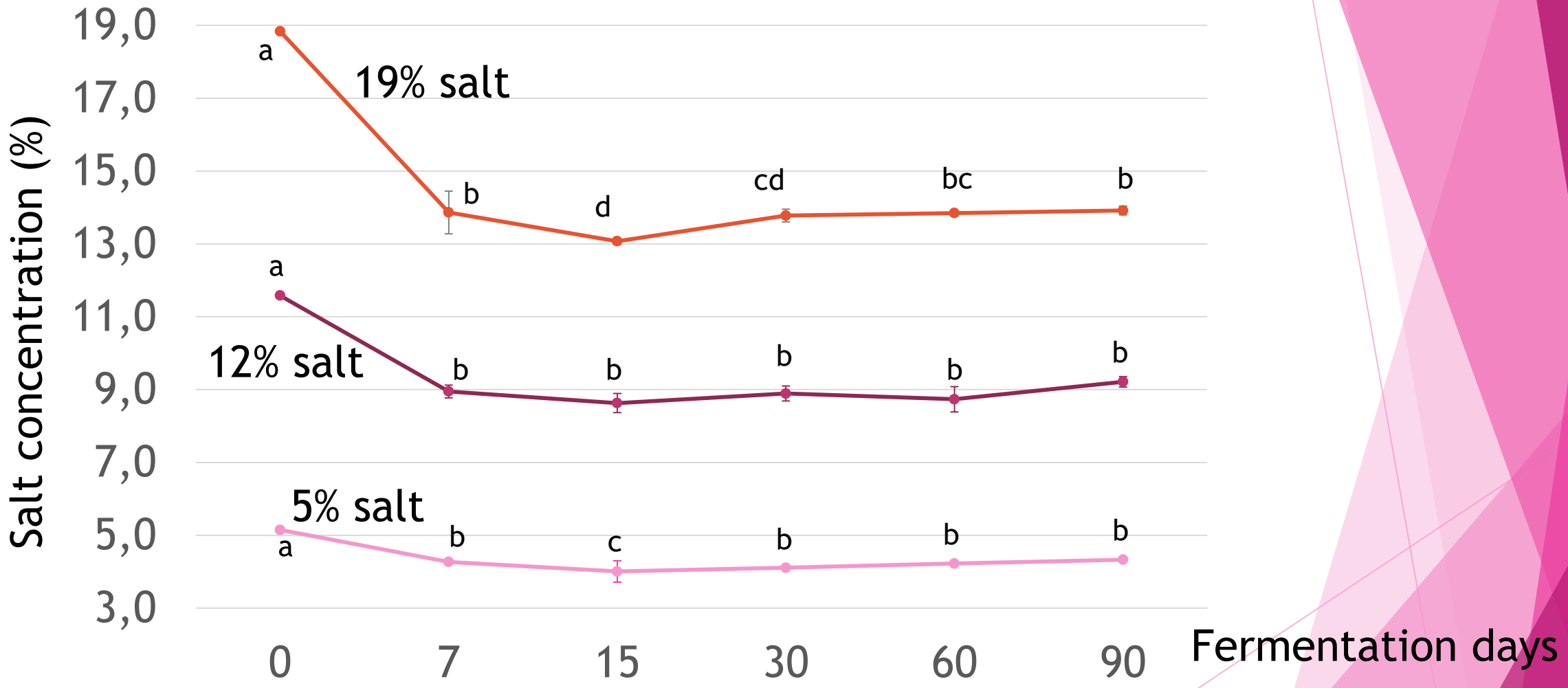
Fermentation days (room temp, 22 °C): 0, 7, 15, 30, 60, 90

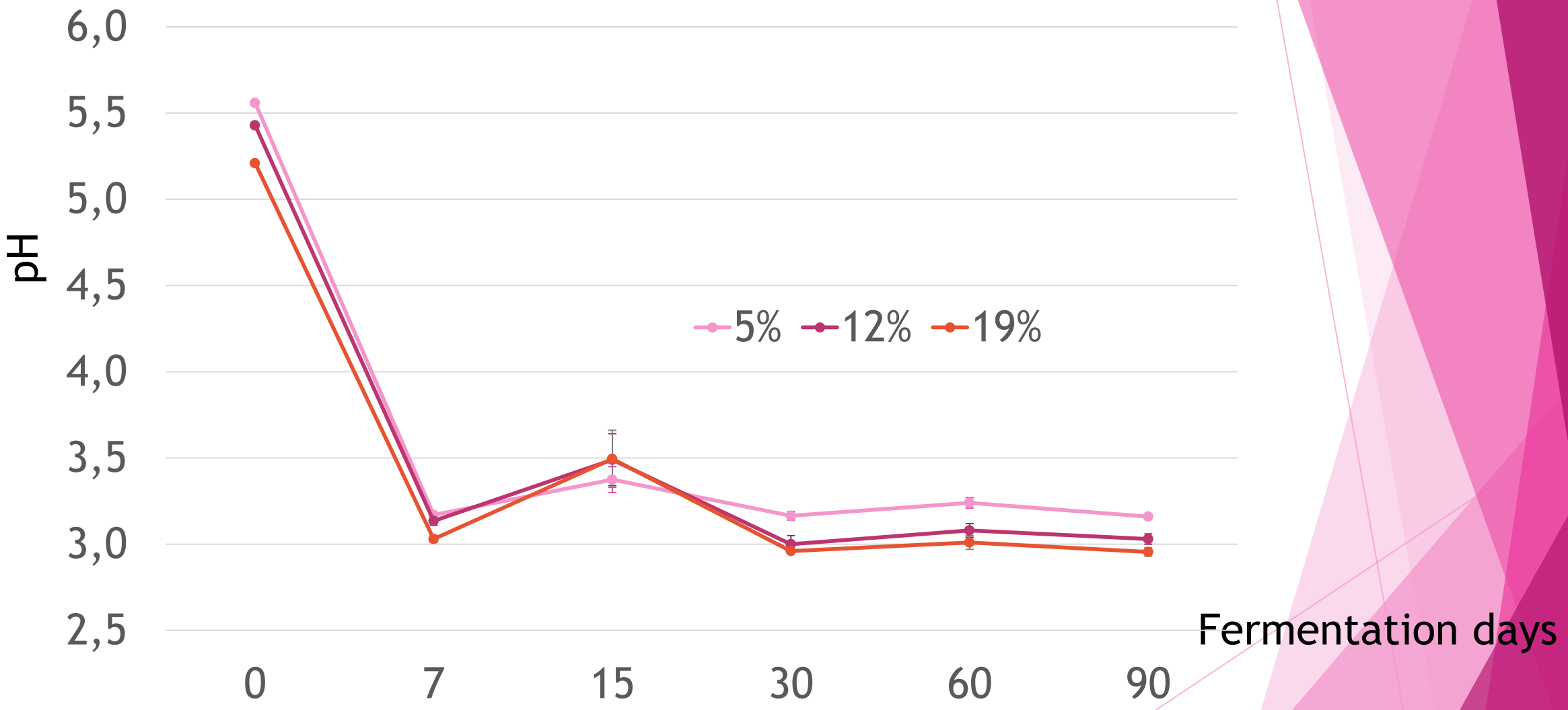
5% SC 12% SC 19% SC

Salt concentration during the fermentation period



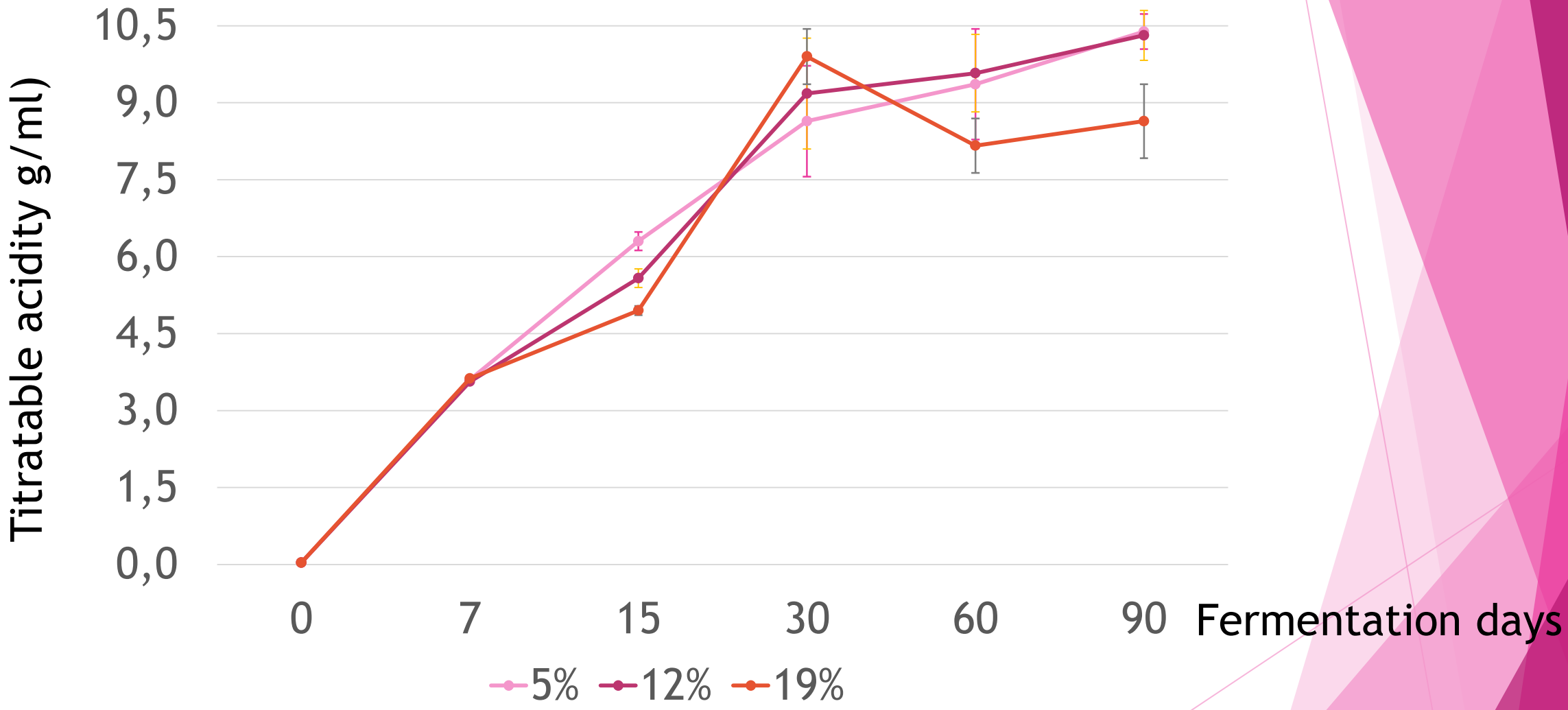
- Determined using the Mohr method (AOAC, 1971)
- The SC showed a significant decrease in the first week.

pH during the fermentation period



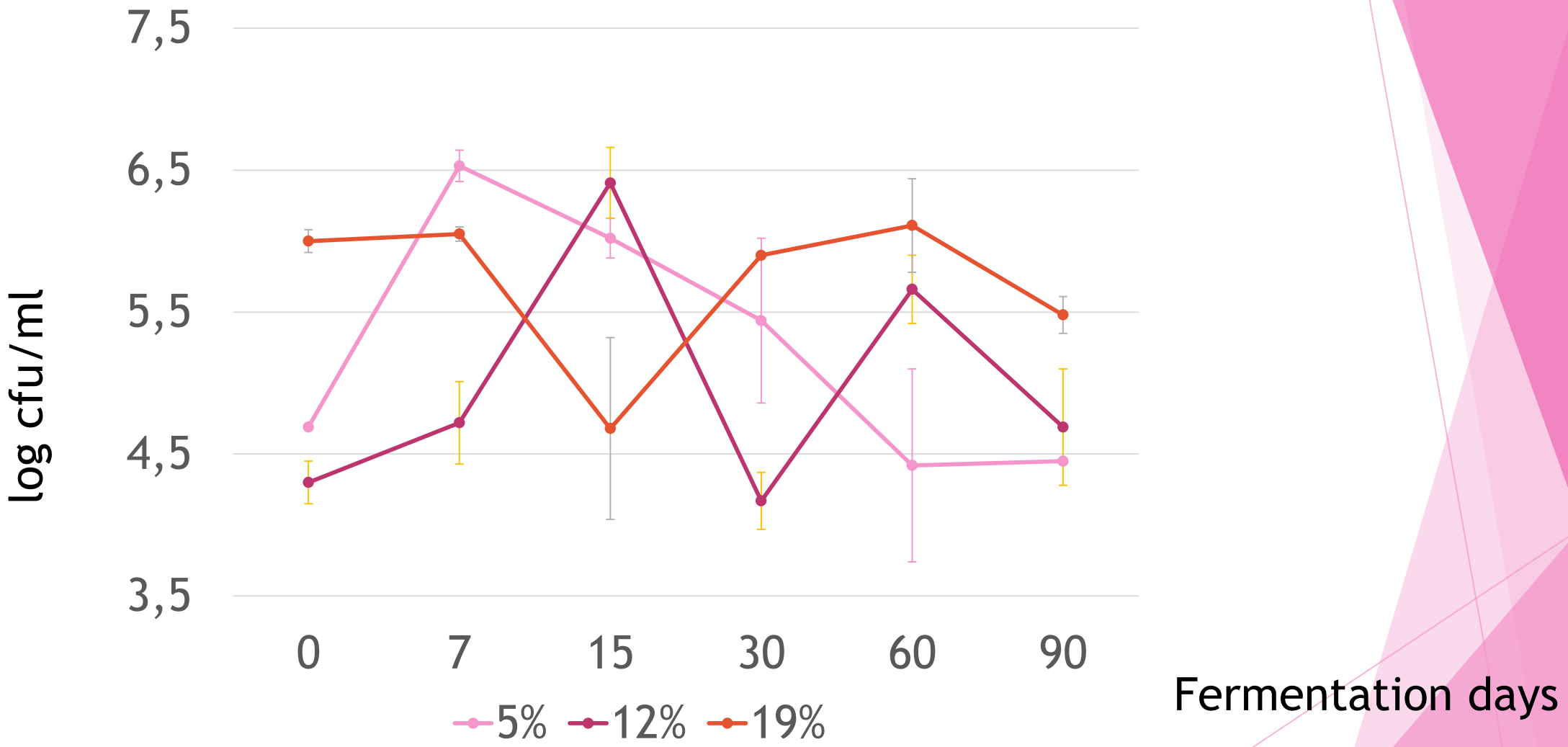
- The pH dramatically decreased during the first week, and remained constant after 30 days in all SC.

Titratable acidity (g/ml)



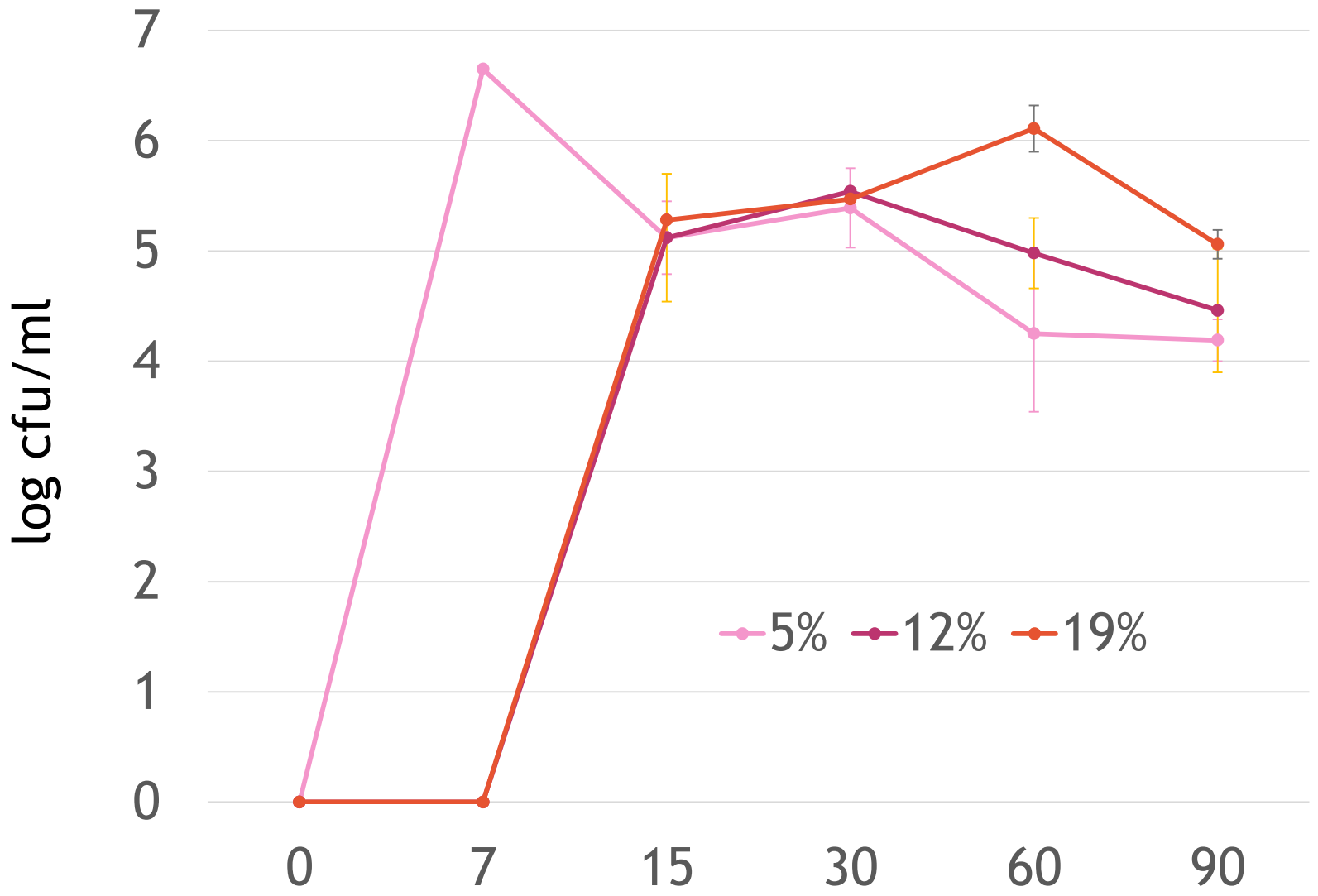
- The titratable acidity increased during the first 30 days and remained relatively constant after that in all SC.

Total mesophilic aerobic bacteria



- PCA, 30°C for 3 days
- Variable during the 90-day period

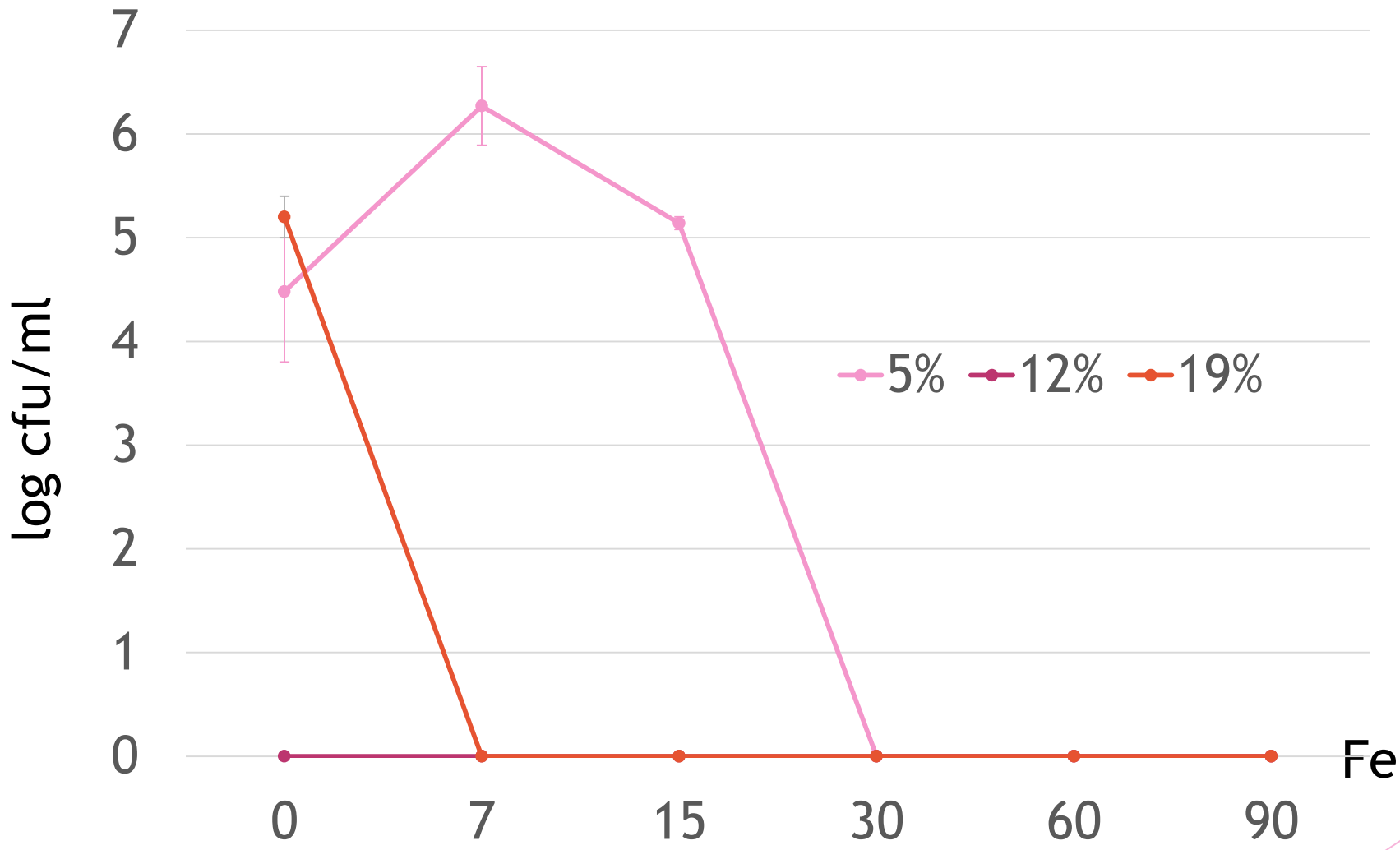
Lactic acid bacteria



Lactic acid bacteria start growing within the first week in 5% SC, but after the first week in 12% and 19% SC.

- MRS, 30°C for 2 days under anaerobic conditions

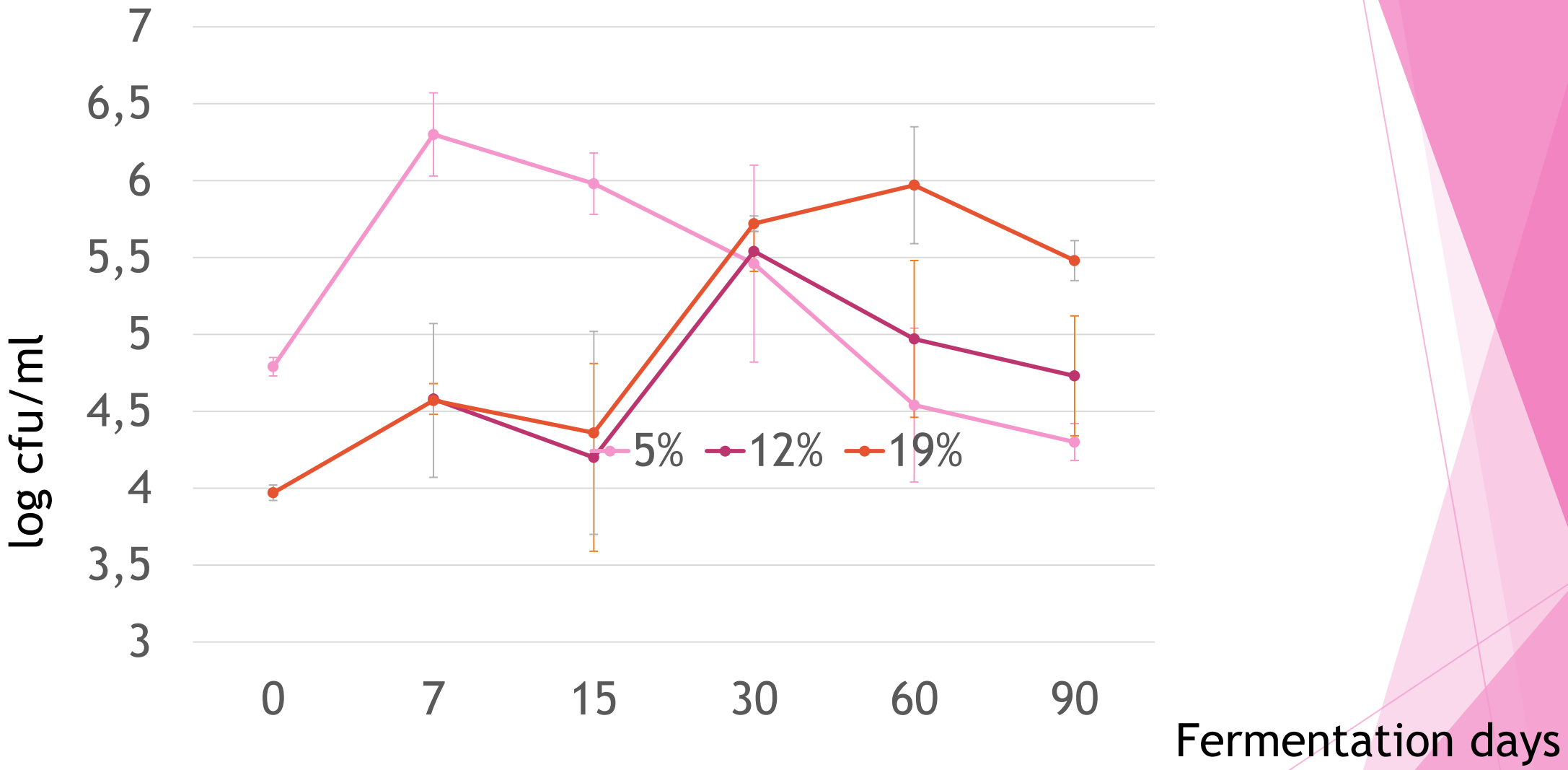
Enterobacteriaceae



Enterobacteriaceae members were not observed after day 7 in 12% and 19% SC, while they seem to have been eliminated after 30 days in 5% SC.

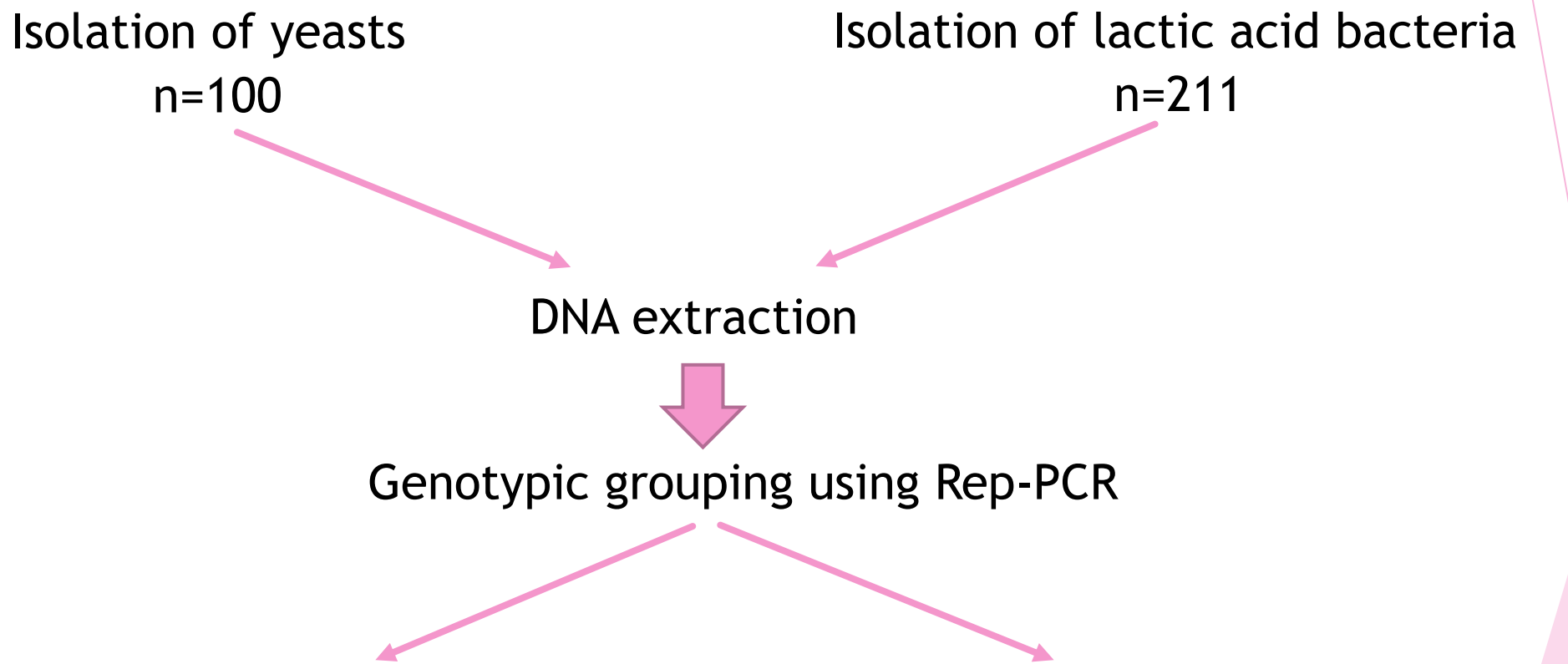
- VRBA, 30°C for 1 day

Yeasts and molds



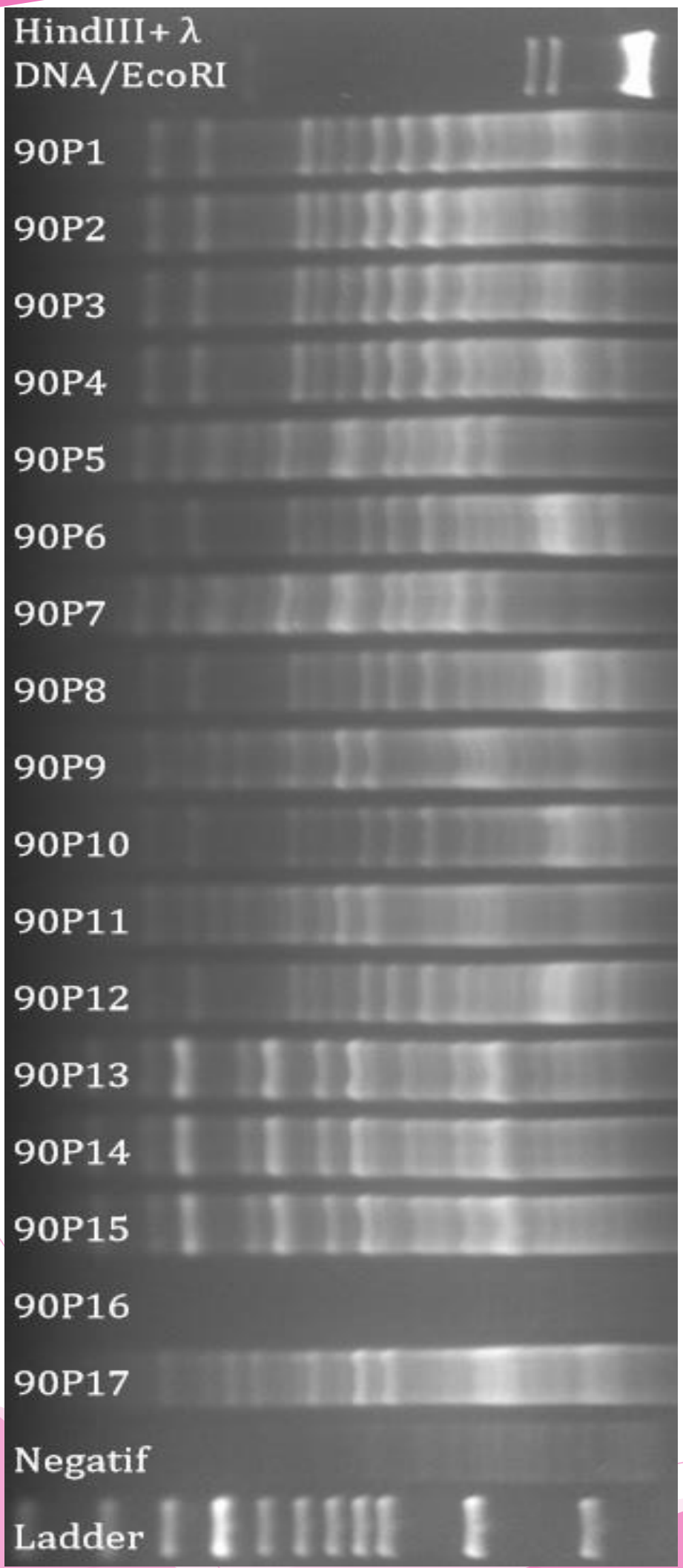
- PDA, 25°C or 5 days
- Variable, cfu/ml numbers are as high as lactic acid bacteria

Yeast and LAB diversity of brine



Diversity of yeasts

- A total of 100 yeasts were isolated from PDA (potato dextrose agar)
- DNA isolation → cell lysis by glass beads followed by chloroform extraction and ethanol precipitation
- Grouping by Rep-PCR
  - Primer: (GTG)5 (5'-GTGGTGGTGGTGGTG-3')
  - PCR conditions: 95°C for 7 min initial denaturation
    90°C for 30 s denaturation
    40°C for 1 min annealing
    65°C for 8 min extension
    65°C for 16 min final extension
- Agarose gel electrophoresis: 1.5% agarose, 30 volts


HindIII+λ
DNA/EcoRI
90P1
90P2
90P3
90P4
90P5
90P6
90P7
90P8
90P9
90P10
90P11
90P12
90P13
90P14
90P15
90P16
90P17
Negatif
Ladder
1
1
1
1
2
1
2
1
3
1
3
1
4
4
4
3

26S rDNA (D1/D2) region sequencing

- Yeasts → 100 isolates → 5 different groups by rep-PCR

↓

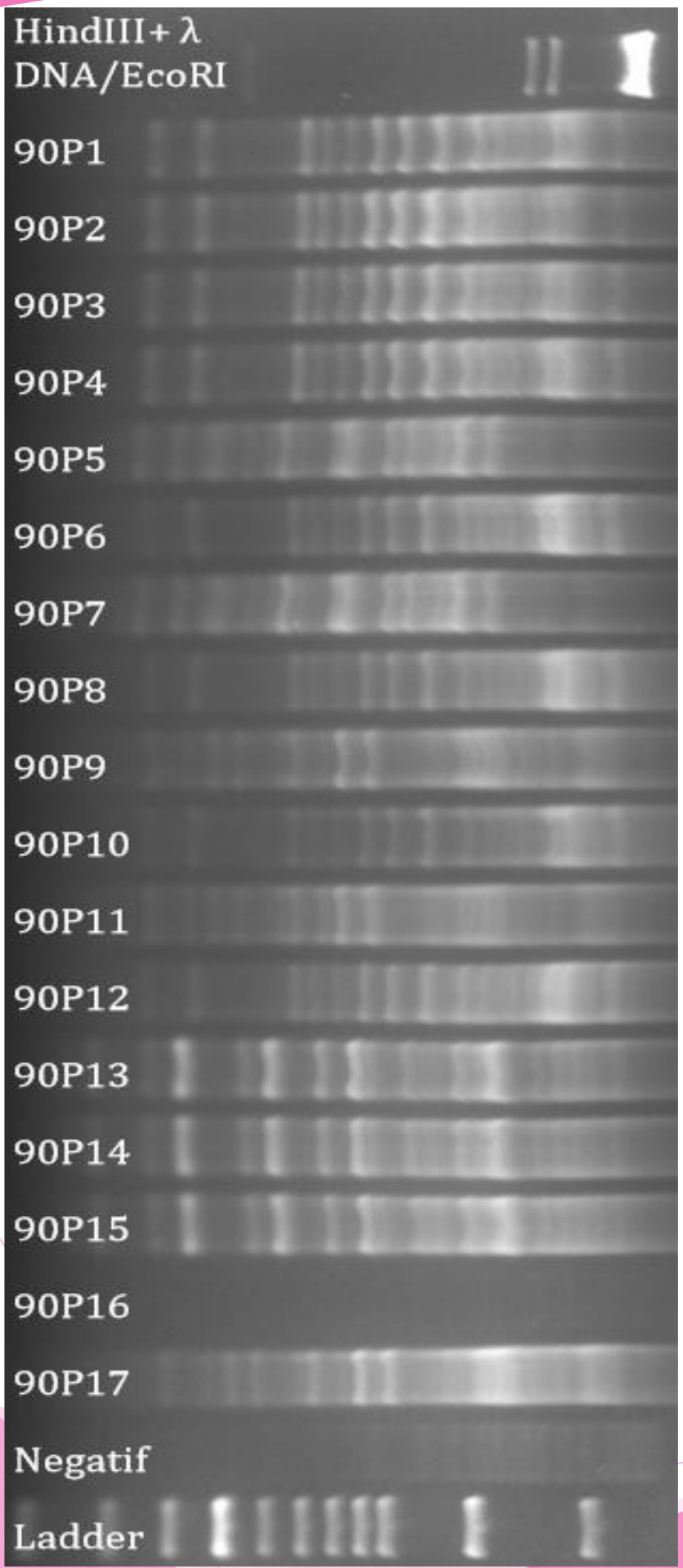
Multiple 26S sequencing
from each group

↓

26S sequencing
performed in 22 isolates

↓

5 different species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90P14
90P15
90P16
90P17
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Ladder
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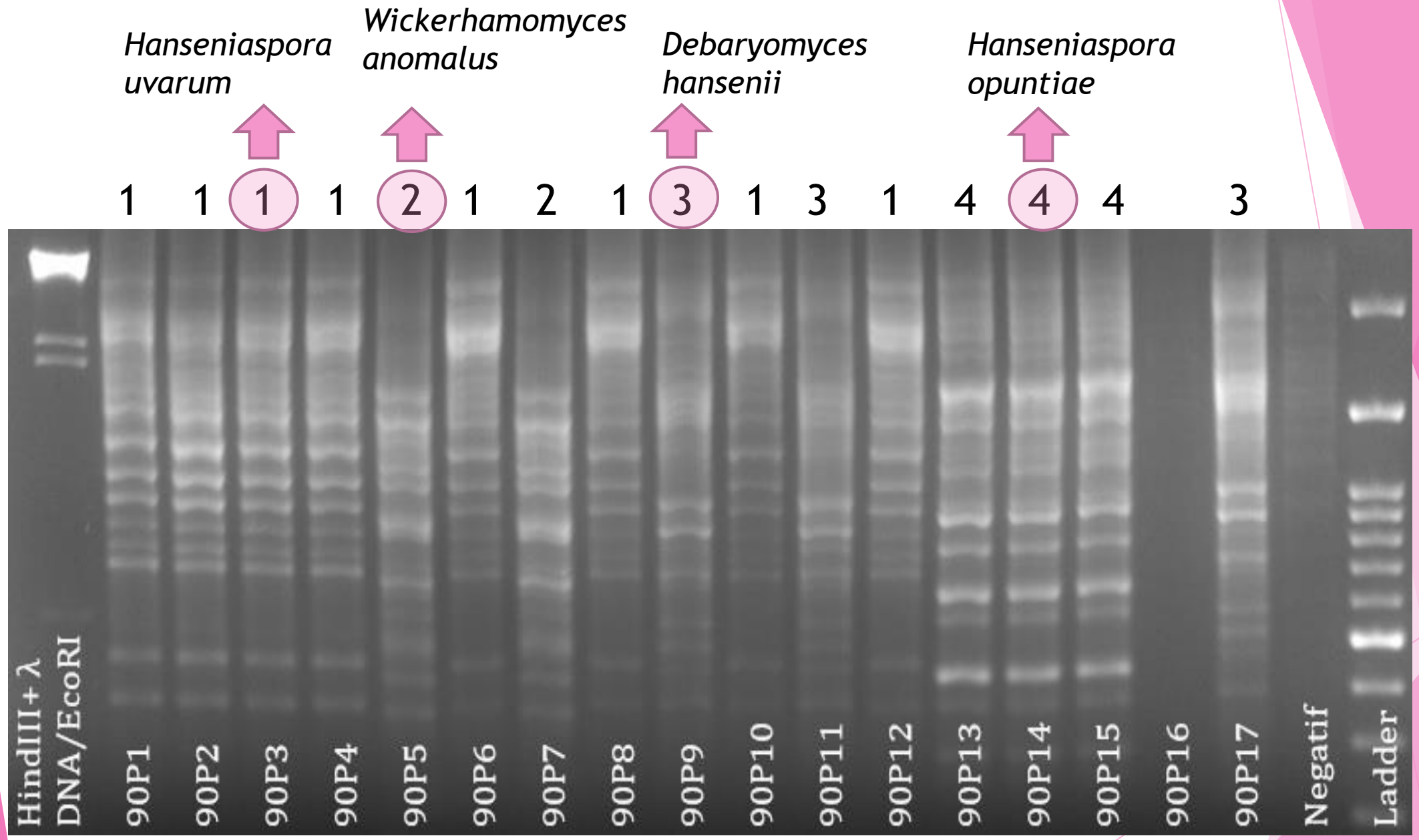
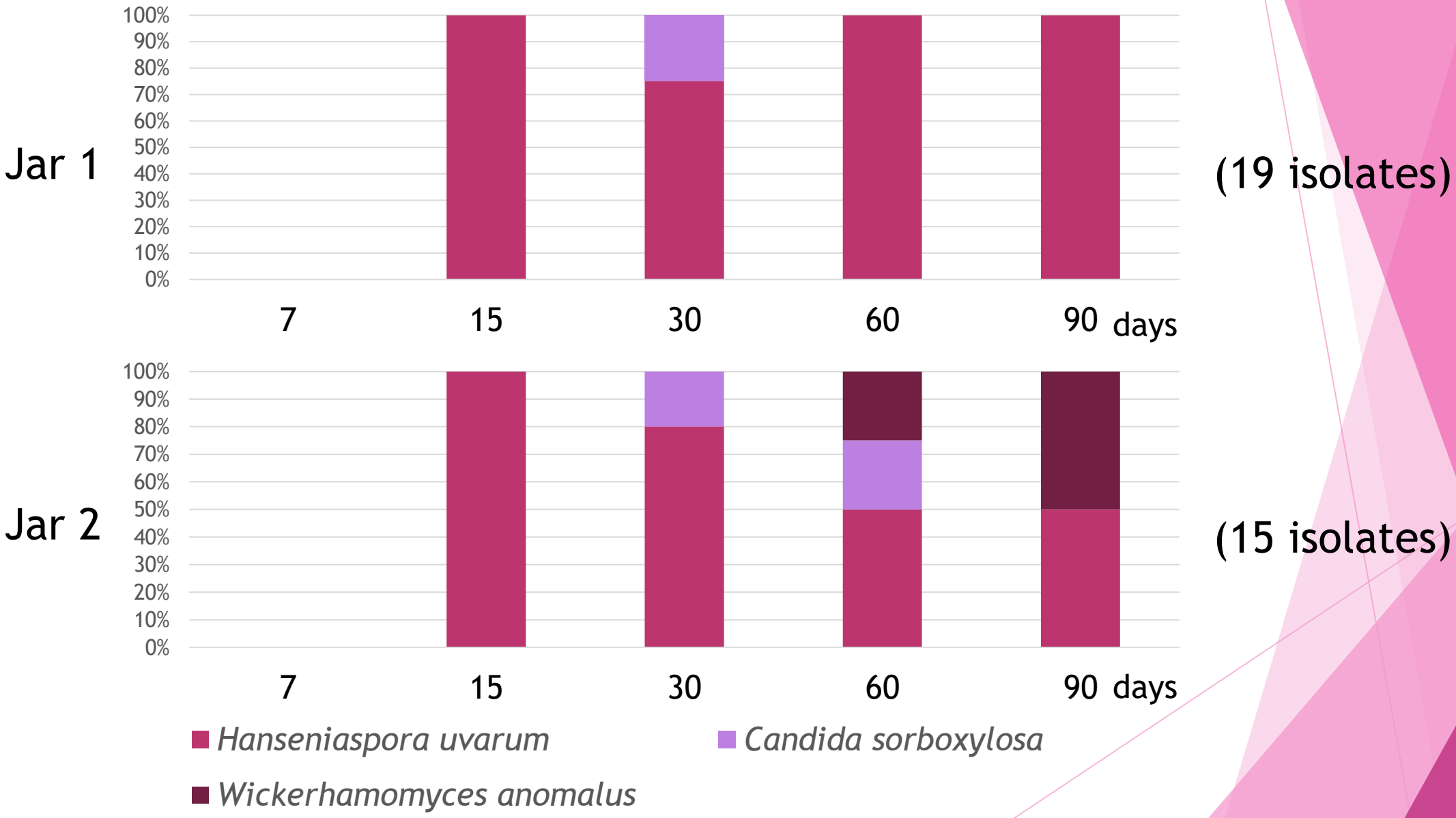
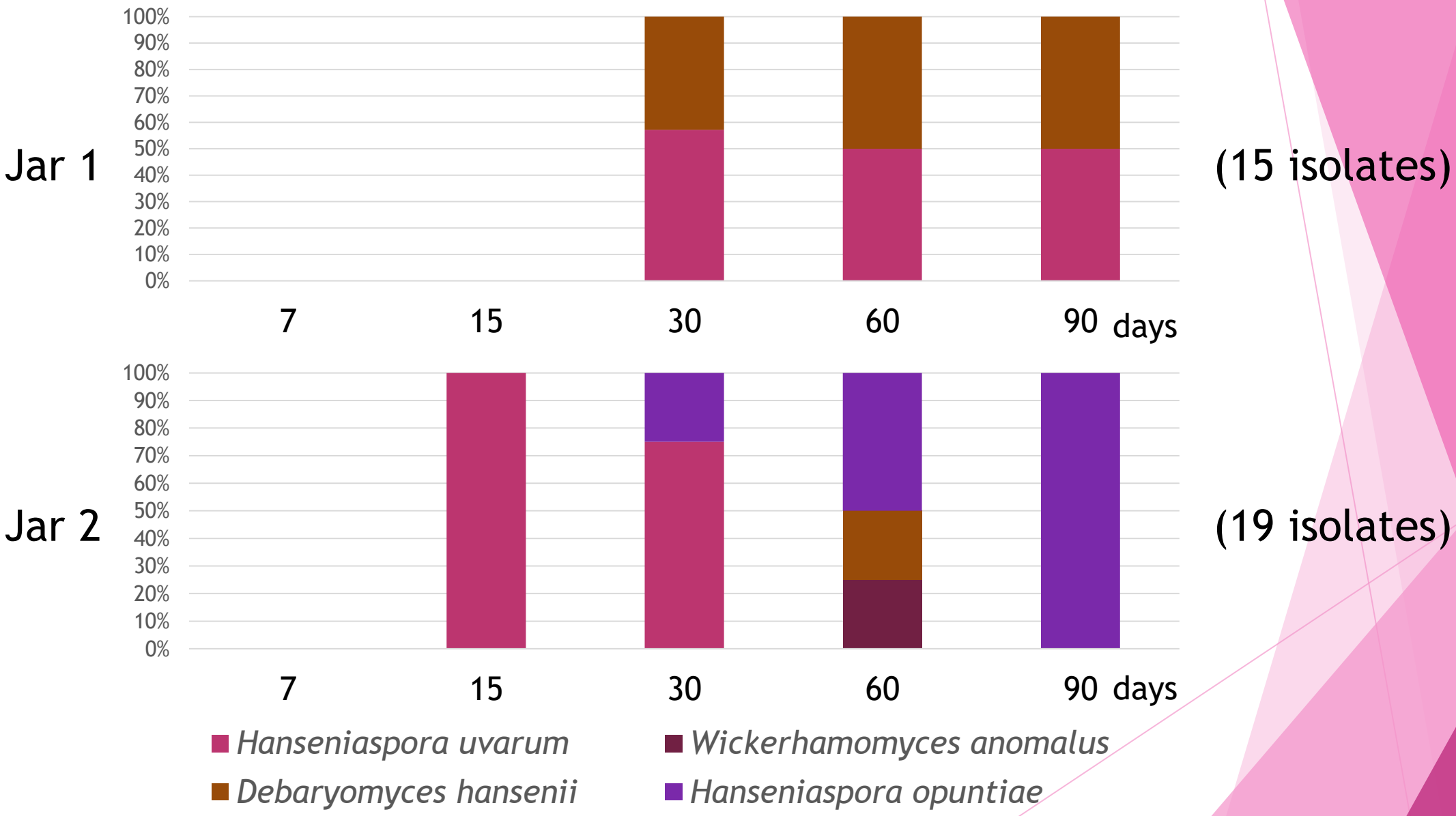

Hanseniaspora uvarum
Wickerhamomyces anomalus
Debaryomyces hansenii
Hanseniaspora opuntiae
1 1 1 1 2 1 2 1 3 1 3 1 4 4 4 3
HindIII+λ DNA/EcoRI
90P1
90P2
90P3
90P4
90P5
90P6
90P7
90P8
90P9
90P10
90P11
90P12
90P13
90P14
90P15
90P16
90P17
Negatif
Ladder

5% salt concentration

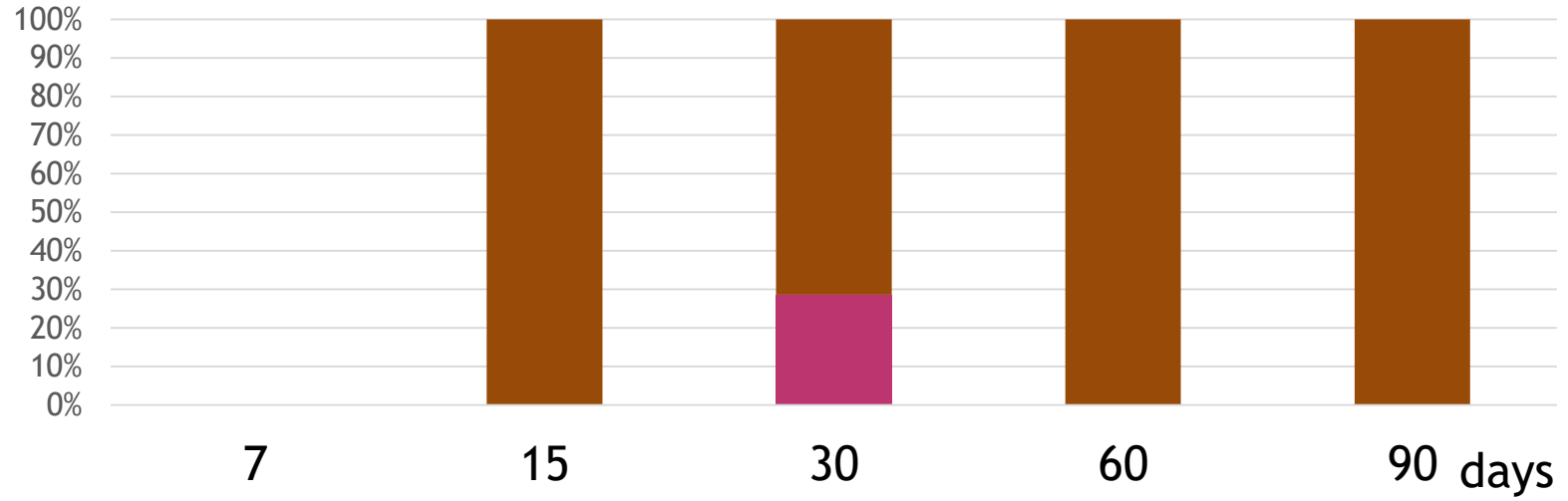


12% salt concentration



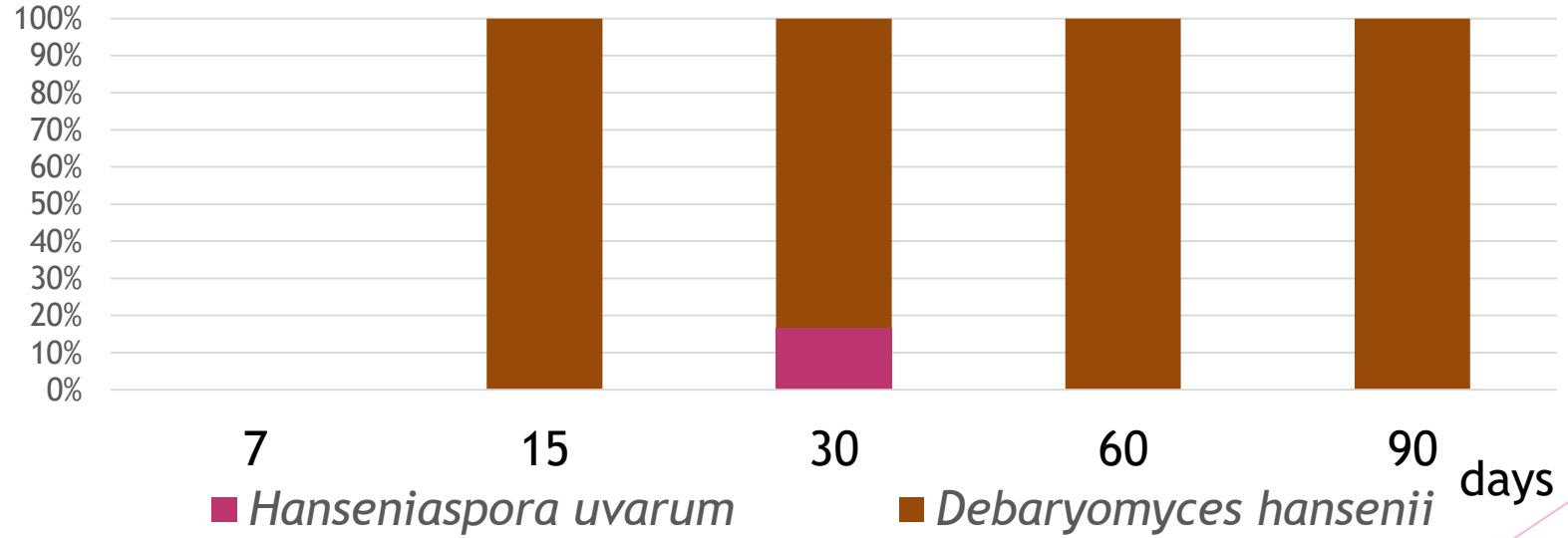
19% salt concentration

Jar 1



(15 isolates)

Jar 2



(17 isolates)

Diversity of LAB

- A total of 211 LAB were isolated from MRS agar
- DNA isolation → lysozyme-based DNA extraction
- Grouping by Rep-PCR
  - Primer: (GTG)5 (5'-GTGGTGGTGGTGGTG-3')
  - PCR conditions: 95°C for 7 min initial denaturation
    90°C for 30 s denaturation
    40°C for 1 min annealing
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- Agarose gel electrophoresis: 1.5% agarose, 30 volts

Main conclusions

- The salt concentration (SC) showed a significant decrease in the first week and remained largely constant after that.
- The pH dramatically decreased during the first week, and remained constant after 30 days in all SC.
- The titratable acidity increased during the first 30 days and remained relatively constant after that in all SC.
- Lactic acid bacteria start growing within the first week in 5% SC, but after the first week in 12% and 19% SC.
- *Enterobacteriaceae* members were not observed after day 7 in 12% and 19% SC, while they seem to have been eliminated after as long as 30 days in 5% SC.
- 5% SC brine was dominated by *Hanseniospora uvarum*.
- 19% SC brine was dominated by *Debaryomyces hansenii*.
- A rather mixed population is observed in the brine with 12% SC

Future studies

- Identification of lactic acid bacteria already grouped by GTG5 rep-PCR
- Analysis of a wider range of processing parameters
- Determination of the relationship of these parameters to microbial communities and quality parameters e.g. flavor, color, texture

Acknowledgment

- The experiments were conducted by MSc student, Esra Nur Yaşa in IZU laboratories.
- Supervisors: Dr. Banu Metin, IZU Dept. of Food Engineering

  Dr. Zeki Durak, YTU Dept. of Food Engineering

Thank you for listening !